PLANT HYBRIDIZATION ALTERS ARTHROPOD COMMUNITY STRUCTURE: PATTERNS OF DIVERSITY AND ABUNDANCE ON PARENTAL AND HYBRID CATTAILS

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PLANT HYBRIDIZATION ALTERS ARTHROPOD COMMUNITY STRUCTURE: PATTERNS OF DIVERSITY AND ABUNDANCE ON PARENTAL AND HYBRID CATTAILS

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Hybridization creates novel genotypes that may differ from the parental species in traits that mediate ecological interactions. In plants, the response of insect communities to hybrid plants is of particular interest, with changes in insect abundance potentially feeding back on plant populations or having impacts on the higher trophic levels that rely on insects for food. In this project, I focused on cattails (*Typha* spp.), which are widespread, dominant wetland plants. In northern North America, the native cattail species *T. latifolia* hybridizes with *T. angustifolia* to form a distinct, vigorous form known as *T.* × *glauca*, which creates dense monocultures via vegetative growth, and is considered invasive. The goal of this research was to determine how arthropod communities respond to this hybrid plant, and to use one important insect species as a case study to uncover mechanisms determining insect abundance in cattail hybrid zones. Chapter 1 describes an extensive survey of the arthropod community assembling around cattail, and shows that diversity and abundance of arthropods is depauperate on the hybrid compared to *T. latifolia*, but

similar to that on *T. angustifolia.* Abundance patterns differed by species, however, and certain important species showed depressed abundance on the hybrid compared to either parental species. One such species is the seed-eating moth *Limnaecia phragmitella*, and Chapters 2 and 3 explore potential mechanisms for why hybrid plants appear to have increased resistance to this herbivore. I show that female moths do not avoid hybrid plants as oviposition sites, and that poor larval performance due to food limitation (from reduced seed set) is the most likely mechanism driving this species' abundance pattern. Since low fertility is common in hybrid plants, low abundance of seed-feeding herbivores probably represents a predictable consequence of hybridization. Chapter 4 discusses identification of hybrid cattails, and presents sets of traits based on genetically-identified cattails that can be used by researchers and managers to distinguish first-generation hybrids from the parental species in the field. Overall, this research provides a valuable new perspective to questions surrounding the effects of hybridization on community ecology, and the role of hybridization in insect-plant interactions.

BIOGRAPHICAL SKETCH

Sarah Joiner Reilly grew up in Framingham, Massachusetts and earned a bachelor's degree in Biology from the College of William and Mary in Virginia in 2001. She worked for a year as a lab assistant at University of Virginia and Mountain Lake Biological Station, and then began her graduate studies at Cornell in 2002. She soon became very involved with her work as a teaching assistant for Introductory Biology, Evolutionary Biology, and Ecology courses. She developed an independent research project following her interests in insect ecology, invasion biology, and community ecology, and developed her own methodologies for working with the species in her chosen study system. She was successful at funding her research, securing grants from both the National Science Foundation and the Cornell Center for the Environment, which allowed her to pursue the molecular components of her project as well as hire undergraduate field assistants. In 2007, Sarah and her husband James (also a graduate student) welcomed a baby boy into their family, and progress on her dissertation slowed. The family grew again in 2008 with the arrival of another boy, and in 2009 Sarah moved to Baton Rouge, Louisiana where her husband had a postdoctoral position. She continued her data analysis and writing from home, while working as a full-time mother. In 2010, a third baby was born, and the family relocated to Virginia. Sarah finished her dissertation work in August 2012. She is currently focused on raising her three boys and sharing with them her fascination with all living things. Although she is not currently part of the professional scientific community, Sarah finds science in everyday life and believes that being a scientist is more than just having a career in science. When the children are older, Sarah hopes to find new opportunities to teach biology, either in traditional classroom settings or through innovative outreach programs.

To all the would-be scientists who put family first.

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CHAPTER 1

PATTERNS OF DIVERSITY AND ABUNDANCE OF ARTHROPODS ON PARENTAL VERSUS HYBRID CATTAILS

ABSTRACT

Human activity is predicted to increase worldwide rates of interspecific hybridization, but the ecological consequences of novel hybrid genotypes are not well understood. Hybrid plants can have altered interactions with insect herbivores, leading to changes in arthropod communities that can have cascading effects on community and ecosystem processes. This study examines patterns of arthropod diversity and abundance on cattails (*Typha* spp.), which are ecologically and economically important wetland plants known for their tendency to hybridize and create dense monocultures via vegetative growth. We conducted an extensive survey to characterize the arthropod communities assembling around parental vs. hybrid cattail plants, including not only herbivores but parasitoids, predators, scavengers, and other members of the broader arthropod community in this system. We examined not only community metrics, but also abundance patterns of individual species to determine whether different arthropod species respond to hybrid plants in similar ways. We found that *T. latifolia*, the broad-leaved cattail, supported greater diversity and abundance of arthropods than *T. angustifolia*, the narrow-leaved cattail, or their F1 hybrid *T.* × *glauca*. Diversity and overall abundance on hybrids was generally similar to *T. angustifolia*, though certain ecologically important species were much less abundant on the hybrid than on the parental species. Unlike the case in some other hybrid systems, cattail hybrids do not support greater biodiversity than parental species. In fact, the hybrid cattail *T.* ×

glauca supports a depauperate arthropod community compared to *T. latifolia*, and this conclusion justifies concern about the increasing prevalence of hybrid cattails in North American wetlands.

INTRODUCTION

Natural hybrid zones are increasingly recognized as scenes of important ecological and evolutionary activity, and as scenes of both genetic diversity and biodiversity (Strauss 1994, Whitham et al. 1999). At the same time, human activity is leading to increasing hybridization between native and non-native species, as a result of species introductions (accidental and intentional) (Mooney and Cleland 2001, Schierenbeck and Ellstrand 2009) and the breakdown of geographic or ecological barriers to gene flow (Abbot et al. 2003). The removal of such barriers can bring closely related species into contact, resulting in hybridization when no intrinsic barriers to reproduction exist.

We know very little about the ecological consequences of hybridization. Hybridization between native and introduced species is predicted to cause losses of biodiversity, as previously distinct entities are homogenized through genetic assimilation (Levin et al. 1996, Rhymer and Simberloff 1996). But the effects of hybridization can extend beyond the parental species to affect community and ecosystem processes. Hybridization is thought to have triggered invasiveness in some plant lineages (Ellstrand et al. 2010, Schierenbeck and Ellstrand 2009). Hybridization also produces novel genotypes that can exist in different ecological habitats than the parental species (Ellstrand et al. 2010, Rieseberg et al. 2003), or interact differently than the parentals with other organisms. For example, hybridization can alter plant characteristics that mediate insect-plant interactions. Hybrid plants often differ from parental species in chemistry (Orians 2000) and plant architecture (Aguilar and Boecklen 1992, Whitham et al. 1999). These

differences can affect the abundance of insect herbivores in hybrid zones (Dungey et al. 2000, Cattell and Stiling 2004, Hochwender and Fritz 2004, Bangert et al. 2006), which in turn can affect the community structure of plants and the higher trophic levels that rely on insects.

Insect responses to hybrid plants determine insect abundance in hybrid zones, and thus strongly influence the ecological consequences of hybridization. There are four basic patterns of insect abundance on hybrid plants relative to the parental species: greater than either parental, less than either parental, intermediate between parentals, and equal to one parental. Boecklen and Spellenberg (1990), Fritz et al. (1994), and subsequent authors (e.g. Strauss 1994, Whitham et al. 1999, Fritz et al. 1999) related the abundance patterns of insect herbivores and plant pathogens specifically to genetic hypotheses for plant resistance mechanisms, with the patterns corresponding to concepts of hybrid susceptibility (outbreeding depression), hybrid resistance (heterosis or hybrid vigor), additive effects, and dominance, respectively.

Reviews of herbivore and pathogen abundance in diverse systems have concluded that all four possible responses occur (Strauss 1994, Whitham et al. 1999). However, in most cases, hybrids appear to support communities with richness and abundance as great or greater than the communities on either parental species. Although some taxa are less abundant or not present on hybrid plants, many hybrids seem to accumulate specialists from both parental species.

Cases where herbivores and pathogens are less abundant in hybrid zones (hybrid resistance) could be considered the most important because they would cause decreased biodiversity and habitat quality in hybrid zones. But cases of hybrid resistance actually appear to be rare: out of 152 cases reviewed, hybrid resistance occurred in only seven (Whitham et al. 1999). However, two-thirds of the 30 plant hybrids included in the review were confined to just a

few genera (*Populus* (9), *Eucalyptus* (6), *Quercus* (5)). The likelihood that a new hybridization event will result in hybrid resistance is not known.

In order to investigate the ecological impact of hybridization in a system where hybrid resistance is known to occur, we conducted a community-level investigation of the arthropods associated with hybrid cattails (*Typha* spp.). *Typha* is an important wetland plant that has been shown to display hybrid resistance to one of its most important specialist herbivores, the moth *Limnaecia phragmitella* (Eisenbach 1996). Specifically, we investigated whether cattails display hybrid resistance to other herbivores besides *Limnaecia*, and whether they more generally support depressed arthropod diversity and abundance that will lead to lower biodiversity in wetlands where hybrid prevalence is increasing (Zedler and Kercher 2004).

METHODS

Study System

Cattails (*Typha* spp.) are dominant wetland plants known for their tendency to hybridize and form monocultures in disturbed wetlands. Two species of cattails occur in northern North America. *Typha latifolia* (TL), the broad-leaved cattail, is native. *Typha angustifolia* (TA), the narrow-leaved cattail, has been presented in many papers as a European introduction (based on Stuckey and Salamon 1987). New evidence from pollen studies suggests that it was present in North America before European settlement, but was not widespread (Shih and Finkelstein 2008). In either case, its range has been expanding (Grace and Harrison 1986, Galatowitsch et al. 1999, Smith 2000, Shih and Finkelstein 2008) and it is considered invasive in many areas. These species hybridize to produce *T.* × *glauca* (TG), a vigorous invasive form apparently capable of out-competing both parental species through vegetative growth (Smith 1987, Waters and Shay

1990, 1992, Galatowitsch et al. 1999, Smith 2000, Zedler and Kercher 2004). There is a diverse arthropod assemblage associated with *Typha* (Claassen 1921, Beaulieu and Wheeler 2002), including leaf-feeders, stem-borers, and many species that reside in the fluff of the senesced seed-heads. This study was conducted in the area surrounding Ithaca, NY (Tompkins County), where there are numerous ponds and marshes dominated by cattails, forming discrete patches that contain various combinations of parental and hybrid cattails. Although *T. angustifolia* is novel in some parts of the United States, it was common in this area in the late 1800s (Dudley 1886), so there has been plenty of time for hybridization to occur. Indeed, hybridization is widespread in most regions where *T. latifolia* and *T. angustifolia* co-occur (Kirk et al. 2011), and while the majority of hybrids appear to be F1s (Kuehn et al. 1999, Snow et al. 2010, Travis et al. 2010, Kirk et al. 2011) several molecular studies have shown that introgression does occasionally occur (Snow et al. 2010, Travis 2010 et al., Kirk et al. 2011). However, the prevalence of introgression varies considerably across sites or regions, with some areas having few or no introgressed individuals (Olson et al 2009, Kirk et al. 2011). Since hybrid class (F1, F2, backcross, etc) has been shown to affect herbivore abundance, we used microsatellite DNA analysis to verify the genotypes used in this study (see below).

Arthropod Sampling

In May and July 2006, we sampled the arthropod communities on cattail plants from 8 sites in and around Ithaca, NY. The May survey targeted arthropods overwintering in mature seed heads and senesced cattail stalks. The second survey, in July, targeted arthropods associated with actively growing and flowering cattails. At each site, we identified sampling areas (Figure 1.A1, Appendix) where only one cattail species was growing, as determined by field characters

(Smith 2000). In general, the cattails were well segregated and we simply avoided areas of overlap where two species grew near each other. Sampling areas covered approximately 200 m^2. In small sites, the sampling area encompassed all the area occupied by a given species.

May Survey

In May 2006, senesced cattail shoots from the previous year were collected haphazardly from each sampling area. We collected 30 shoots of each species at each site, or as many shoots as possible if there were fewer than 30 shoots remaining with seed heads still attached (Table 1.1).

Table 1.1. Number of senesced shoots collected from each site.

Site	*T. latifolia*	*T. × glauca*	*T. angustifolia*
A-lot Patch (AL)	31	0	30
Cayuga Marsh (CM)	17	30	0
Guthrie Patch (GU)	25	30	30
Mud Pond (MP)	60	30	30
Ostman Pond (OS)	30	31	32
Research Ponds (RP)	31	30	30
Teeter Pond (TP)	27	30	0
Travis Pond (TR)	30	30	11

Seed heads were separated from stems and put in Ziploc bags, and stems were bagged in plastic tubing. The tubing was rolled at the top and bottom and secured using staples at the bottom and a binder clip at the top (so that the bag could be opened and closed again easily). Heads and stems were hung in the laboratory, with natural photoperiod. There was no air conditioning, but fans helped cool the room on particularly hot days.

Bags were checked for arthropods every 1-2 days from the date of collection in late May through the beginning of September. The moths *Limnaecia phragmitella* (Cosmopterigidae), *Dicymolomia julianalis* (Crambidae), and various parasitoid wasps were collected upon emergence and frozen. Spiders residing in the fluff were collected when they were discovered.

Other insects, such as beetles and Lygaeid bugs, crawled out of the fluff and accumulated in the bags. These were collected periodically. At the end of the summer, all the seed heads were thoroughly dissected and all remaining arthropods were collected. All arthropods not frozen were stored in ethanol.

July survey

In July 2006, we collected all cattail shoots (flowering and vegetative) within ten 0.5 x 0.5 m quadrats in each sampling area. The quadrats were distributed approximately 10 m apart along transects. Because the regions varied considerably in shape, the number and location of transects required to obtain ten quadrats varied. The plants were measured (height, number of leaves) and dissected. The presence and extent of herbivore damage was noted, and all insects were collected and stored in ethanol. Leaf tissue was collected from one plant in each quadrat for genotyping and frozen at -20 C. Since cattails are perennial plants, we are confident that the genotypes sampled in July 2006 are also representative of the genotypes growing the previous year that were sampled as senesced plants in May 2006, since the morphologies of plants in each sampling area were unchanged from one year to the next.

Genotyping

Tissue samples were lyophilized and then ground using a GenoGrinder. Ground tissue was returned to –20 C until extraction. DNA was extracted with plant DNeasy kits (Qiagen, Inc. City State), using the basic miniprep protocol. We used 7 primers developed for one of the parental cattail species, *T. angustifolia* (TA3, TA5, TA7, TA8, TA16, TA20) (Tsyuko-Omeltchenko et al. 2003) and tested in North American cattails (Snow et al. 2010). These

primers amplify in *T. latifolia* as well, and appeared likely to amplify distinct alleles in the two parental species in our study populations. F1 individuals would be characterized by having one allele from each parental species at each locus. Advanced hybrids and backcrosses would display a combination of species-specific loci and mixed-species loci. The probabilities of misidentifying F2 or first-generation backcrosses using 6 loci, assuming Mendelian inheritance, are given in Table 1.2. Based on these probabilities, we determined that using 6 alleles was sufficient.

Table 1.2. Probabilities associated with misidentification of backcross or F2 individuals based on 6 microsatellite loci.

Actual Genotype	Apparent Genotype	Probability
Backcross	Pure parental	0.016
Backcross	F1	0.016
F2	Pure parental	0.0005
F2	F1	0.016

The 5' end of each primer was fluorescently labeled with NED (TA3, TA20), VIC (TA5, TA16), 6FAM (TA7) or PET (TA8). PCR was performed using Multiplex PCR kits (Qiagen, Inc City State), using the standard protocol with Q solution, except that the ratios of the primers in the primer mix were optimized (volume per reaction was increased from 0.2 to 0.3 µl for TA5, and decreased to 0.15 for TA16 and TA20). PCR was performed using the following cycling parameters: 95° for 15 min; 7 x (94° C for 30s, 57° C for 1 min 30s (-1° C per cycle)); 72° C for 1 min; 25 x (94° C for 30s, 50° C for 1 min 30s, 72° C for 1 min; 72° C for 10 min. Genotyping was performed using an ABI capillary sequencer in the Evolutionary Genetics Core Facility at Cornell University. Data were collected and scored manually using Genemapper v. 3.0.

Diversity Analysis

To examine patterns of arthropod diversity across *T. latifolia, T. angustifolia,* and the hybrid, we used sample-based rarefaction curves (species accumulation curves) and rank abundance plots.

We used Estimate S (Colwell 2009) to calculate sample-based rarefaction curves for the May and July surveys. These curves represent the expected number of detected species (S) as a function of sampling effort. In the May survey, we sampled as a function of plant. In the July survey, we sampled from quadrats and constructed two sets of curves, one using plant as the sampling unit and the other using quadrat.

We used R (R Development Core Team 2012) to create rank abundance plots using resampling to account for the higher numbers of TL and TG plants sampled in the study. For the May survey, the lowest sample size (TA) was 166 plants, so we resampled all three species based on a sample of 150. For the July survey, the lowest sample size (TL) was 331 plants, so we resampled based on a sample of 300. For both surveys, we resampled 500 times and used the average abundance for each arthropod species to create plots of abundance rank versus abundance per plant. We did not use proportional abundance; therefore the curves cannot be used to make inferences about evenness. Because the abundance of the entire community is substantially higher on TL, comparisons of evenness are not biologically meaningful.

Species Abundance Analysis

Of the 127 arthropod species identified, 45 were found in sufficient abundance to analyze patterns of abundance on hybrid and parental cattails. The abundance of each of these arthropod species was analyzed separately using a mixed effects model with site as a random factor and

cattail species as a fixed effect. For the July survey, the model also had the random factor of quadrat nested within site. Although the abundance patterns of some arthropod species did vary by site, we did not fit a site-species interaction in any model because the purpose of this analysis was to look at the broader effect of plant species across many sites. For species with count data (most species), we used a Poisson distribution. For species with presence/absence data we used a binomial. In the July survey, there were some herbivore damage metrics used as proxies for insect presence. These were continuous, and we used a normal distribution. The analyses were performed in R using the LMER function in the lme4 package (Bates et al. 2011). We used a model selection approach to evaluate the effect of cattail species (TA, TG, and TL) on arthropod abundance. Five models were compared:

1) The null model, with only the random factor and no cattail species effects. Since the model does not try to fit any species information to the data, it represents the hypothesis that for arthropod abundance, TA=TG=TL.

2) The full model, with all three cattail species effects included. This model represents the hypothesis that abundances on TA, TG, and TL are all different.

3) A reduced model, including the effect of TA but lumping the effects of TG and TL. This model represents the hypothesis that abundance on TA is different from TL and TG, and TL=TG.

4) A reduced model, including the effect of TG but lumping the effects of TA and TL. This model represents the hypothesis that abundance on TG is different from TA and TL, and TA=TL.

5). A reduced model, including the effect of TL but lumping the effects of TA and TG. This model represents the hypothesis that abundance on TL is different from TA and TG, and TA=TG.

These five models represent all possible scenarios with respect to cattail species effects. For model comparison we used standard maximum likelihood (not restricted maximum likelihood) which is recommended when comparing models with different fixed effect structures (Pinheiro and Bates 2000, Bolker et al. 2009). Model comparisons were made using delta AIC values and Akaike weights. Akaike weights give the probability that a given model is the best supported of the models being compared. Since we compared all five possible models, this probability is particularly useful. When more than one of the 5 models had some support, we summed the Akaike weights for components of the models that were in common, to draw a less specific but better supported conclusion (Burnham and Anderson 2002).

We also performed a similar analysis designed to determine whether the abundance of each arthropod species on the hybrid was more similar to TL or TA. We included only model 3 (TG=TA) and model 5 (TG=TL), so in this case the Akaike weight gives the probability that the hybrid abundance is more similar one parental than the other.

RESULTS

Genotyping

A total of 218 shoots was genotyped (60 TA, 68 TG, 90 TL), revealing a total of 74 genets (unique genotypes from the same site: 20 TA, 16 TG, and 38 TL). All alleles were species-specific and allele sizes corresponded adequately to previous work (Tsyuko-Omeltchenko et al. 2003, Snow et al. 2010; Table 1.3). All putative parental shoots in this study displayed single-species alleles at each locus. All hybrid plants displayed a clear F1 pattern with one TL allele and one TA allele for each locus. No evidence of introgression was detected in this sample.

Table 1.3. Allele sizes and numbers of alleles found in *T. angustifolia* (20 genets), *T.* × *glauca* (16 genets), and *T. latifolia* (38 genets). All *T.* × *glauca* genets were F1s.

Locus 3			
Allele size (bp)	TA	TG	TL
174			2
176		11	63
178		1	4
180		4	7
209	40	15	
215		1	

Locus 5			
Allele size (bp)	TA	TG	TL
280		15	72
282		1	4
288	15	9	
290	9	3	
294	16	4	

Locus 7			
Allele size (bp)	TA	TG	TL
190		16	65
192			11
196	33	15	
209	7	1	

Locus 8			
Allele size (bp)	TA	TG	TL
270		4	12
272		12	64
276	16	6	
290	3	3	
292	21	7	

Locus 16			
Allele size (bp)	TA	TG	TL
180		16	42
182			31
184			3
194	40	13	
196		3	

Locus 20			
Allele size (bp)	TA	TG	TL
90		1	
92		14	68
94		1	8
100	19	2	
102	11	12	
104	10	2	

Arthropod survey

We found a total of 127 arthropod species from 13 orders associated with cattail plants. (See Table 1.A1, Appendix). In May, the majority of the species collected were Hymenopterans, Lepidopterans, or spiders, but Hemiptera and Lepidoptera were the most abundant taxa (Figure

1.1, a and b). In July, the majority of species were Coleopterans and Dipterans, but most Diptera and Lepidoptera were the most abundant taxa (Figure 1.1, c and d).

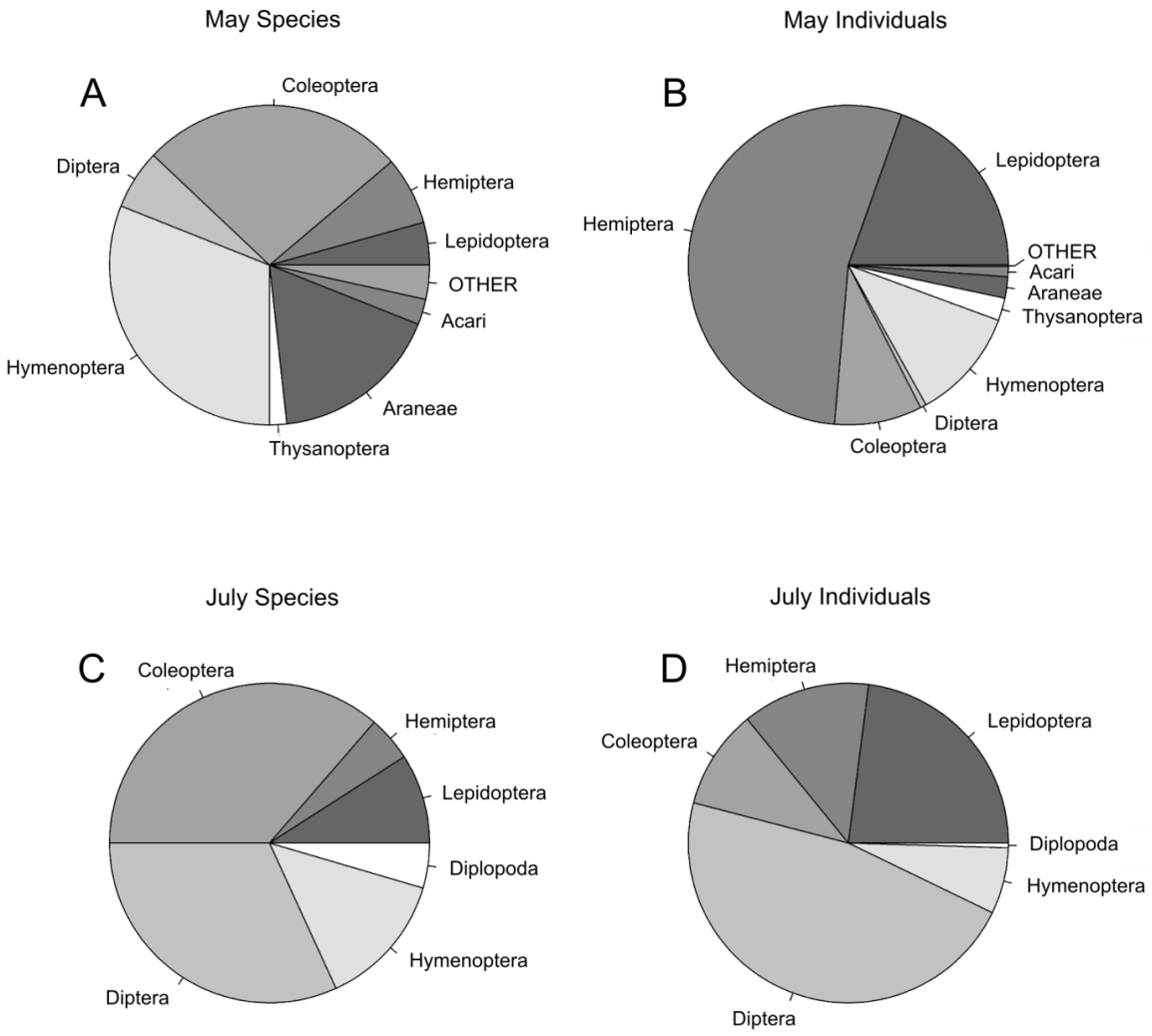

Figure 1.1. Composition of arthropod communities on cattails in May versus July. Charts of individuals represent relative abundance, whereas charts of species represent relative richness by taxon.

Diversity analysis

Both diversity analyses (species accumulation curves and rank abundance plots; Figures 1.2 and 1.3) show that TL consistently supports greater species richness and abundance. In May

(senesced plants and seed heads), the arthropod community on the hybrid cattail is very similar to that on TA, but in July (green plants), the community on the hybrid is intermediate in diversity between the two parental species.

For May, the species accumulation curves for TA and TG are similar, and the curve for TL is higher (Figure 1.2a). This indicates that for every plant sampled, more arthropod species are found on TL than on TA or the hybrid, which is very similar to TA. The relationship is consistent at every sampling level. In contrast, the equivalent curves for July (Figure 1.2b) show the hybrid as intermediate in richness between TL and TA. This intermediate pattern holds regardless of whether the curves are a function of plant or quadrat (Figure 1.2, b and c), implying that the higher species richness seen on TL is not an artifact of TL plants occurring at lower densities (i.e. if a TL and TA quadrat contained the same abundance of arthropods, but the TL arthropods were divided among fewer plants, then sampling by plant would show greater species accumulation on TL whereas sampling by quadrat would show TL and TA equal).

The rank abundance plots are consistent with the patterns seen in the rarefaction curves. In both May and July, TL has greater species richness and the species are more abundant (Figure 1.3). In May (Figure 1.3a), the community on the hybrid is similar to that on TA, whereas in July (Figure 1.3b) the community on the hybrid is intermediate.

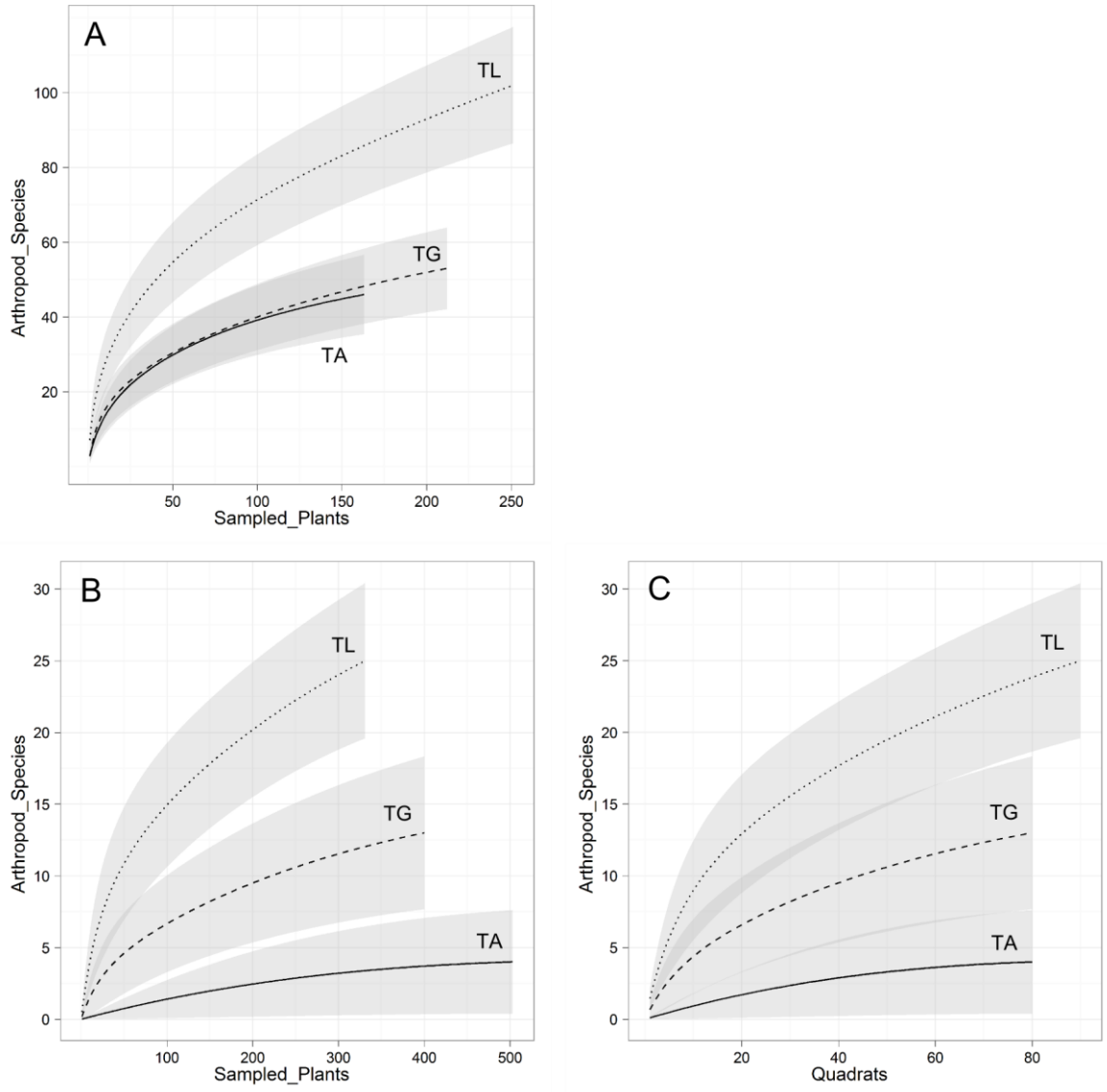

Figure 1.2. Species accumulation curves for May and July. The dotted line is *T. latifolia*, the dashed line is *T. × glauca* , and the solid line is *T. angustifolia*.

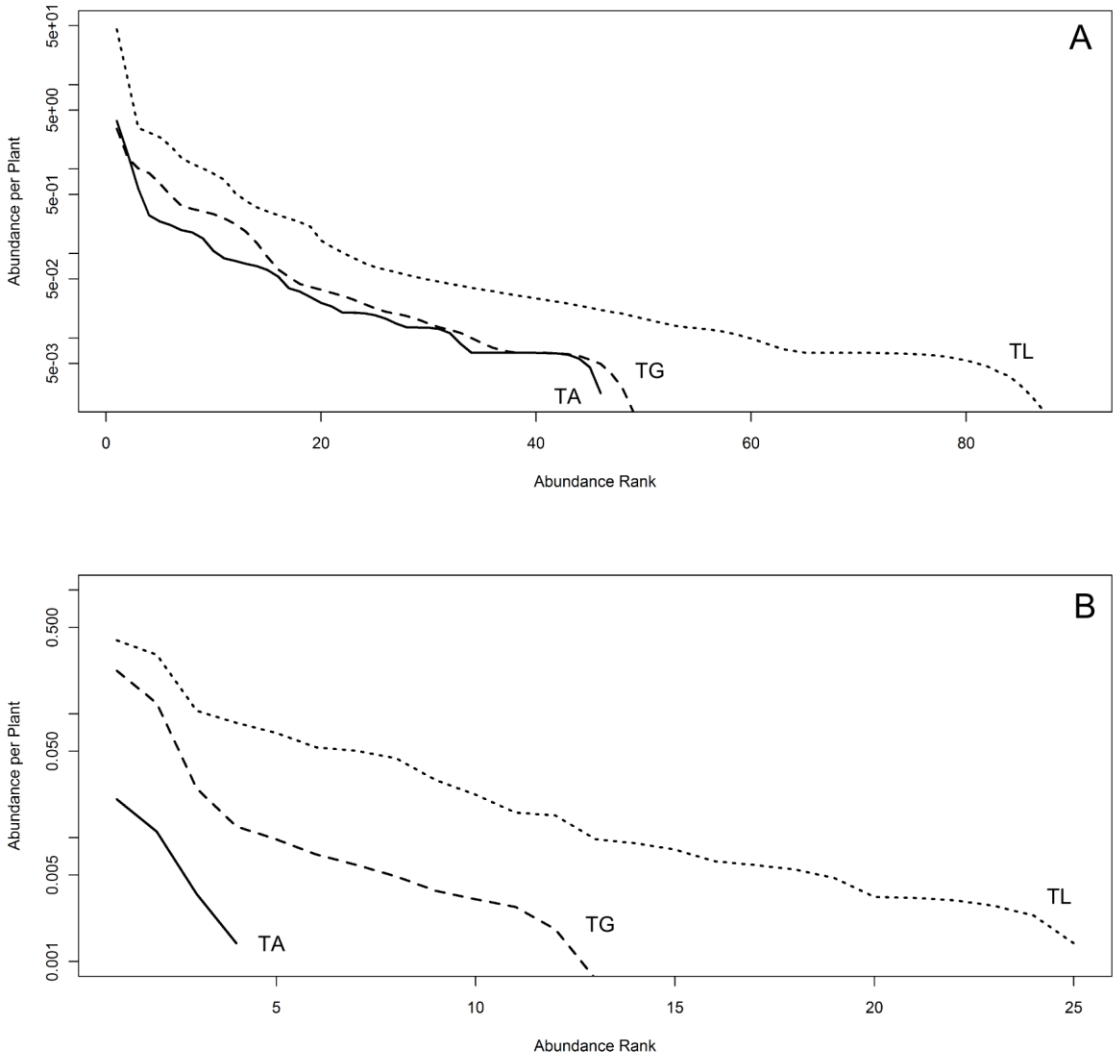

Figure 1.3. Rank abundance curves for (a) May and (b) July. The dotted line is *T. latifolia*, the dashed line is *T. × glauca* , and the solid line is *T. angustifolia*.

Species abundance analysis

We used the results of the abundance analyses to categorize arthropod abundance patterns and identify cases where abundance on the hybrid was elevated, depressed, intermediate, or equivalent to one parental (Table 1.4). For each arthropod species, we used Akaike weights to select the abundance pattern that is best supported (see example, Box 1.1). Abundance patterns for individual species are plotted in Figure 1.A2 (Appendix).

BOX 1.1. Analyzing abundance patterns by model selection

As an example, consider the parasitoid wasp *Macroteleia* (Table 1.2). Based on AIC values, the best-supported model was abundance on all species different (TL ≠ TG ≠ TA), with an Akaike weight of 0.769. This means that there is a 76.9% probability that this is indeed the best model of the set considered. This level of support would generally be interpreted as suggestive, but would not be considered strong evidence in favor of that model. The next best model was TG different from TA and TL, with TL= TA. The difference between the AIC value for this model and that of the best model (delta AIC) was 2.4. The rule of thumb is that a model within 2 of the best model has substantial support; a difference of 4-7 indicates considerably less support, and a difference of greater than 10 indicates essentially no support (Burnham and Anderson 2002). In this case, the existence of a competing model with delta AIC close to two results in the relatively weak Akaike weight for the best model. However, both the top two models have one component in common: that TG is different from the other two species. Summing the Akaike weights for the top two models (0.769 + 0.231=1) gives the probability that either of those models is the best model, i.e. the probability that TG is indeed different from the other two species. Thus in this case, we have very strong evidence that TG is different, and we can use that to conclude confidently that *Macroteleia* follows an elevated abundance pattern, but whether TA and TL are different is much less certain.

Over all species analyzed, we found intermediate and TA-equivalent patterns to be the most common (Figure 1.4). In May, there were some cases of depressed and elevated abundance on the hybrid, but cases of TL-equivalence were virtually non-existent.

The results of the analysis using only two alternative models (TG=TA vs. TG=TL) showed that for most arthropod species, abundance on the hybrid was more similar to abundance on TA than on TL (Table 1.5). Of 42 species in May, 30 had substantial support for the TG=TA model, whereas only 5 had substantial support for the TG=TL model. For 7 other species, the

models were either indistinguishable or lacking strong support for one model over the other. All seven species in July had substantial support for the TG=TA model, as did 5 out of 6 damage metrics.

When abundance patterns were analyzed by functional group, herbivores and predators showed depressed abundance on the hybrid in the May survey, whereas detritivores, omnivores, and fungivores showed intermediate abundance (Table 1.6). The data for parasitoids did not support any particular model, but there was strong evidence at least that they do not show elevated abundance on the hybrid. In July, predators, herbivores, and detritivores showed TA-equivalent or intermediate patterns on hybrids. In both surveys, the pattern for predators was the same as the pattern for herbivores, though the patterns differed between May and July.

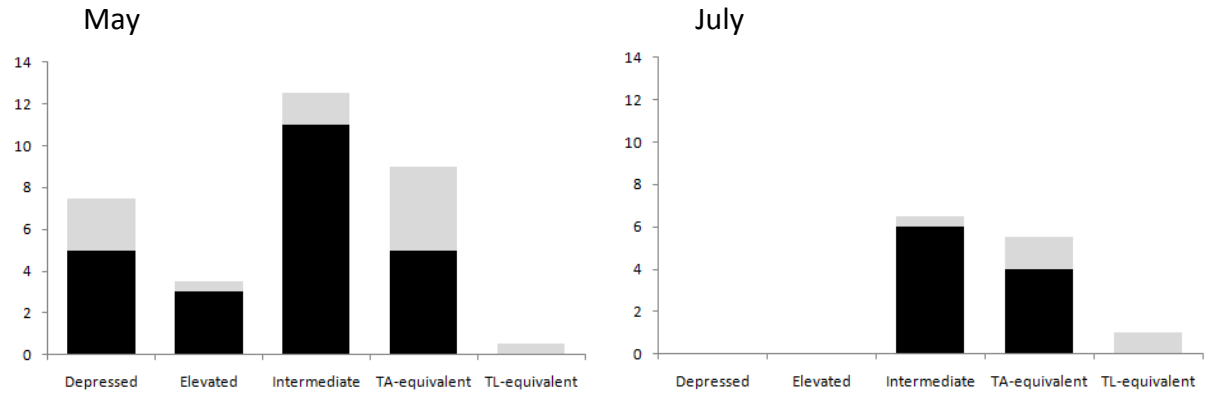

Figure 1.4. Number of species assigned to each abundance pattern in the May and July surveys. We used black and gray to represent different strengths of support for each species' category assignment (from Table 1.2). The black portion of the bar represents the number of species with Akaike weight (w) > 0.9. The gray portion represents the number of species with w < 0.9. For species where two models were equally supported, we assigned half a point to each abundance category.

Table 1.4. Results of analysis of arthropod abundance from the May and July surveys. Shown here are the Akaike weights (w) associated with the best individual model and the best summed model, and the conclusion regarding distribution pattern. A star (*) indicates conclusions with w < 0.9, which should be treated as tentative.

Arthropod Species	N	Best individual model: w	Best summed model: w	Abundance on Hybrid
May Survey				
Limnaecia phragmitella	3870	all different: 1	-	Depressed
Dicymolomia julianalis	789	all different: 1	-	Intermediate
*Chilacis typhae (*adult)	1721	all different: 0.988	TL different: 1	Intermediate
*Chilacis typhae (*juv)	10491	all different: 1	-	Depressed
*Kleidocerys residae (*adult)	15	TA = TG, TL diff: 0.652	TL different: 0.98	TA-equivalent or Intermediate
*Kleidocerys residae (*juv)	619	all different: 1	-	Depressed
Orthoperus sp.	861	all different: 0.999	TL different: 1	Intermediate
Melanopthalma sp.	307	all different 0.928	TL different: 1	Intermediate
Sapintus fulvipes	397	all different: 0.612	TL different: 1	Intermediate or TA-equivalent
Sapintus lemniscatus	425	all different: 1	-	Intermediate
Phalacrid beetle (unknown sp.)	14	TA=TG, TL diff: 0.365	TA = TG: 0.573	Inconclusive
Biphyllid beetle (unknown sp.)	51	TA=TL, TG diff: 426	TG ≠ TL: 0.996, TG different: 0.583	Inconclusive (TG>TL)
Telephanus sp.	11	TA=TG, TL diff: 0.529	TL ≠ TG: 0.795, TL different: 0.732	TA-equivalent or Intermediate*
Cecidomyiid fly (unknown sp.)	99	TA=TG, TL diff: 0.734	TL different: 1	TA-equivalent*
Stratiomyiid fly (adult)	14	TG=TL, TA diff: 0.435	TA different: 0.732	TL-equivalent*
Eristalis sp. (adult)	19	all different: 0.506	TL different: 0.999	Intermediate or TA-equivalent
Elachiptera sp.	10	TA=TG, TL diff: 0.719	TL different: 0.98	TA-equivalent*
Hymenochaonia sp.	89	TA=TG, TL diff: 0.733	TL different: 1	TA-equivalent*
Temelucha gracilipes	88	all different: 0.752	TL different: 1	Intermediate*
Scambus sp. D1	29	TA=TG, TL diff: 0.341	TA = TG: 0.589	Inconclusive
Chelonus sp.	131	all different 0.630	TL different: 1	Intermediate or TA-equivalent
Apanteles sp.	101	all different: 0.962	TL different: 1	Depressed
Macroteleia sp.	91	all different 0.769	TG different: 1	Elevated
Eupelmus sp.	71	all different: 0.770	TL different: 1	Depressed*
Chalcedoid Q1	224	TA=TL, TG diff: 0.725	TG different: 0.999	Depressed
Chalcedoid Q2	154	all different: 1	-	Depressed
Chalcedoid Q3	514	all different: 1	-	Depressed
Chalcedoid Q4	57	TA=TG, TL diff: 0.525	TL different: 0.809	Inconclusive
Chalcedoid Q7	24	all different: 0.613	TL different: 0.999	Depressed or TA-equivalent
Wasp R4	10	TA=TG, TL diff: 0.732	TL different: 0.998	TA-equivalent
Wasp U	23	all different: 0.628	TL different: 0.997	Intermediate or TA-equivalent

Table 1.4. (Continued)

Wasp AB	57	TA=TL, TG diff: 0.674	TG different: 1	Elevated
Spider B1	15	TA=TL. TG diff: 0.402	TG different: 0.680	Depressed*
Sitticus sp.	107	TA= TL, TG diff: 0.685	TG different: 1	Depressed
Spider A2	15	TA=TG, TL diff: 0.540	TL different: 0.976	TA-equivalent or Intermediate
Thysanoptera (unknown thrips sp.)	545	all different: 1.0	-	Intermediate
Mite (unknown non-physogastric sp.)	229	all different: 1.0	-	Elevated
Physogastric mite (unknown sp.)	40	TA=TG, TL diff: 0.621	TL different: 0.999	TA-equivalent or Depressed
Tettigoniid grasshopper (unknown sp.)	17	all equal: 0.440	TA = TL: 0.611	Inconclusive
Psocid (unknown barklouse sp.)	12	TG=TL, TA diff: 0.330	TG ≠ TA: 0.858, TA different: 0.578	TL-equivalent or Elevated*
Spider C3	25	all equal: 0.321		Inconclusive
Spider C4	23	all equal: 0.312		Inconclusive
Spider A1	66	all equal: 0.349		Inconclusive
Spider A3	32	all equal: 0.393	TA=TL: 0.605	Inconclusive
Wasp AA	10	TA=TG, TL diff: 0.409	TL ≠ TG: 0.854, TL different: 0.615	TA-equivalent or depressed*
July Survey				
Species:				
Bellura oblique	39	TA=TG, TL diff: 0.693	TL different: 0.968	TA-equivalent*
Archanara oblonga	9	TA=TG, TL diff: 0.561	TL different: 0.969	TA-equivalent or Intermediate
Stratiomyid (larvae)	39	TA=TG, TL diff: 0.666	TL different: 0.994	TA-equivalent
Eristalis sp. (larvae)	18	TA=TG, TL diff: 0.653	TL different: 0.984	TA-equivalent or Intermediate
Rhynchophorus pertinax typhae	2	-	-	Inconclusive (both individuals on TL)
Scymnus sp.	10	TA=TG, TL diff: 0.483	TL ≠ TG: 1.0	Inconclusive (TL>TG)
Ulidiid fly (unknown sp.)	27	TA=TG, TL diff: 0.417	TL different: 0.800	TA-equivalent or Intermediate*
Aphidae (unknown sp., binary)	27	all different: 0.502	TL different: 0.942	Depressed or TA-equivalent
Damage metrics:				
Archanara 1st instar leaf cuts	10	TA=TG, TL diff: 0.537	TL different: 0.979	TA-equivalent or Intermediate
Bellura/Archanara stem holes	186	all different: 1.0	-	Intermediate
Bellura/Archanara burrows (binary)	135	all different: 1.0	-	Intermediate
Central leaves dying (binary)	66	all different: 0.540	TL different: 0.967	Intermediate or TA-equivalent
Chewed leaf centers (centimeters)	43	all different: 1.0	-	Intermediate
Chewed leaf edges (centimeters)	65	TG=TL, TA diff: 0.712	TA different: 1	TL-equivalent*
All herbivore damage (binary)	243	all different: 1.0	-	Intermediate

Table 1.5. Results of analysis to determine whether the abundance of each arthropod species on the hybrid is more similar to *T. angustifolia* or *T. latifolia*. Indicated with X for each species is the model (TG=TA or TG=TL) that received the most support, and the corresponding Akaike weight (w). In some cases, neither model received strong support. Where there was some evidence that one model was better but the support was not strong, + is used instead of X.

Arthropod Species	N	TG similar to:		w
May Survey		TA	TL	
Limnaecia phragmitella	3870	X		1
Dicymolomia julianalis	789	X		1
*Chilacis typhae*_adult	1721	X		1
*Chilacis typhae*_juv	10491	X		1
*Kleidocerys residae*_adult	15	X		0.995
*Kleidocerys residae*_juv	619	X		1
Psocid	12		X	0.896
Tettigoniid	17	-	-	0.507
Orthoperus sp.	861	X		1
Melanopthalma sp.	307	X		1
Sapintus lemniscatus	425		X	1
Sapintus fulvipes	397	X		1
Phalacrid	14	-	-	0.668
Telephanus	11	X		0.999
Biphyllid	51	X		0.998
Stratiomyiid	14	-	+	0.736
Eristalis sp.	19	X		0.999
Cecidomyiid (unknown sp.)	99	X		1
Elachiptera sp.	10	X		0.978
Ichneumonid (unknown sp.)	88	X		1
Hymenochaonia sp.	89	X		1
Macroteleia sp.	91		X	0.999
Chelonus sp.	131	X		1
Apanteles sp.	101	X		1
Eupelmus sp.	71	X		1
Chalcedoid Q1Q4	281	X		1
Chalcedoid_Q2Q3	668	X		1
Chalcedoid_Q7	24	X		1
Wasp_R4	10	X		0.999
Wasp_U	23	X		0.992

Table 1.5. (Continued)

Wasp_AA	10	X		0.901
Wasp_AB	57	X		1
Thrips_primary	545		X	1
Spider A1	66	-	-	0.636
Spider_A2	15	X		0.971
Spider A3	32	-	-	0.514
Spider B1	15	X		0.958
Spider_C1C2	107	X		0.999
Spider C3	25	-	-	0.666
Spider C4	23	-	-	0.61
Physogastric mite	40	X		1
Mite_primary	229		X	1
July Survey				
Bellura obliqua	39	X		1
Archanara oblonga	9	X		0.954
First_instar_*Archanara*_damage	10	X		0.975
Holes in stem (from borers)	186	X		1
All Borer damage (binary)	135	X		1
Center leaves dying (binary)	66	X		0.93
Eristalis sp.	18	X		0.999
Stratiomyid	39	X		1
Scymnus sp.	10	X		1
Ottidae	27	X		0.996
Aphids_binary	27	X		1
Chewed leaf edges (cm)	65		X	1
Chewed leaf center (cm)	43	X		0.997
All Herbivore damage (binary)	243	+	-	0.81

Table 1.6. Results of analysis of arthropod abundance from the May and July surveys, based on functional group. Shown here are the Akaike weights (w) associated with the best individual model and the best summed model, and the conclusion regarding distribution pattern. A star (*) indicates conclusions with w < 0.9, which should be treated as tentative.

Functional group	N	No. species	Best individual model: w	Best summed model: w	Abundance on Hybrid
May					
Herbivores	17307	17	all different: 1.0	-	Depressed
Detritivores	46	5	all different: 0.832	TL different: 1	Intermediate*
Fungivores	2080	11	all different: 1.0	-	Intermediate
Omnivores	790	2	all different: 1.0	-	Intermediate
Predators	345	26	TA= TL, TG diff: 0.625	TG different: 0.999	Depressed
Parasitoids	1728	31	all different: 0.571	TL different: 1	Not elevated
July					
Herbivores	57	4	TA=TG, TL diff: 0.717	TL different: 1.0	TA-equivalent*
Detritivores	65	5	TA=TG, TL diff: 0.569	TL different: 1.0	TA-equivalent or Intermediate
Predators	20	6	TA=TG, TL diff: 0.618	TL different: 0.871	TA-equivalent or Intermediate*
Parasitoids	11	2	TA=TG, TL diff: 0.442	TL ≠ TG: 0.999	inconclusive (TL>TG)

DISCUSSION

Many hybrid zone studies consider only herbivores on hybrid plants because the intent is to use herbivore abundance on hybrids to make inferences about genetic mechanisms for plant resistance traits (Fritz et al. 1994, Fritz et al. 1999; Whitham et al. 1999, Hallgren et al. 2003, Hochwender et al. 2000, Nahrung et al. 2009). The purpose of our study was to evaluate the ecological consequences of hybridization by assessing the arthropod communities that assemble around parental vs. hybrid plants, regardless of whether the interaction of a particular species with the plant is direct or indirect. A few studies have investigated tritrophic interactions among hybridizing plants, herbivorous insects, and their parasitoids (Eisenbach 1996, Cattell and Stiling 2004). Wimp et al. (2004) conducted a broader survey of arthropods on *Populus* hybrids (herbivores, parasitoids, predators, etc), but we are the first to examine the responses of individual species in an arthropod community to hybrid vs. parental host plants.

We found that cattail hybrid zones (*T.* × *glauca or* TG) generally support depauperate arthropod communities, compared to the parental species *T. latifolia* (TL). The arthropod community on the hybrid was generally similar to or somewhat higher in diversity and abundance than the corresponding community on the other parental, *T. angustifolia* (TA), even though genotyping results revealed that most plants in the study were F1 hybrids. Thus in this system, the hybrid does not support communities with greater biodiversity than either parental species, unlike the scenario described for some other hybrid systems (Whitham 1989, Whitham et al. 1999). Previous studies that reported greater biodiversity in hybrid zones attributed that increase to the ability of specialist herbivores from both parental species to utilize hybrid hosts (Whitham 1989, Whitham et al. 1999). In our study, we encountered relatively few arthropods

associated with only one parental. With a few exceptions, the species encountered in this study were found on both parentals, though their abundances usually differed markedly.

Consistent with previous studies (reviewed by Strauss 1994, Whitham et al. 1999), we found that different arthropod taxa respond differently to the hybrid plant in this system. We found that all possible responses to the hybrid did occur, but the most common pattern was one in which abundance on the hybrid was intermediate between the parentals and more similar to *T. angustifolia* than *T. latifolia*. In most of these cases, the analysis revealed equally strong support for the intermediate and TA-equivalent models. We found some examples of both elevated and depressed abundance on the hybrid relative to the parental, but very few cases of TL-equivalence. In most cases, *T. angustifolia* was the more resistant parental species, making cases of TA-equivalence examples of "dominance of resistance" (*sensu* Fritz et al. 1999). The prevalence of such cases implies that *T. angustifolia* may possess many dominant traits relevant to arthropod abundance.

Analyzing abundance patterns by functional group revealed interesting patterns. In May, herbivores and predators both showed depressed abundance. The predators are primarily jumping spiders, and they are probably feeding largely on *Limnaecia phragmitella* larvae (personal observation), which are highly abundant overall and show depressed abundance on the hybrid. Detritivores, omnivores, and fungivores were found in intermediate abundance on the hybrid. The fungivores were composed primarily of minute beetles (primarily *Orthoperus*, *Sapintus*, and *Melanopthalma*), which made up a large portion of the arthropod community in *T.* × *glauca* seed heads. Little is known of the fungal communities associated with cattail, but diverse fungal assemblages have been described on *T. latifolia* (Pugh and Mulder 1971). The parasitoid responses were varied, which is not surprising since many of them probably follow the

abundance patterns of their hosts (though in some systems parasitism rates have been shown to differ among herbivores on parental vs. hybrid host plants, e.g. Cattell and Stiling 2004, Eisenbach 1996). In our study, *Apanteles* is an exclusive parasitoid of *L. phragmitella*, and follows the *L. phragmitella* pattern of depressed abundance on the hybrid.

In contrast, the predators, herbivores, and detritivores in July showed intermediate or TA-equivalent abundance on the hybrid. The herbivores were primarily aphids and the stem boring caterpillars *Archanara oblonga* and *Bellura obliqua*, which displayed an intermediate pattern. The borers were important drivers of diversity in this system, because their burrows, which were filled with decaying plant material and frass, accumulated detritivores and the predators likely feeding upon them.

Overall, the factors influencing arthropod abundance on parental and hybrid cattails must be varied, as the lifestyles of arthropods in our study are highly diverse. There is a paucity of studies that actually compare physiological traits and ecological effects of *T.* × *glauca* to those of the parental cattail species. Most *Typha* ecology studies compare *T. latifolia* to *T. angustifolia* (e.g. Grace and Wetzel 1981a, 1982, 1998), *T.* × *glauca* to a non-*Typha* community (e.g. Woo and Zedler 2002, Boers et al. 2007, Angeloni et al. 2006, Tuchman et al. 2009), or a mix of *Typha* species to a non-*Typha* community (e.g. Wilcox et al. 2008, Vaccaro et al. 2009, Farrell et al. 2010, Mitchell et al. 2011).

The two parental species differ from each other in many traits that are likely to affect arthropod abundance. In morphology, *T. latifolia* has wider leaves and larger pistillate spikes (e.g. Hotchkiss and Dozier 1945, Fassett and Calhoun 1952, Smith 1967, Grace and Harrison 1986, Kuehn and White 1999), and more and larger seeds (Marsh 1962, Grace 1985). Its stems are considerably broader and lack the tough central core that characterizes *T. angustifolia*

(Galatowitsch et al. 1999). *T.* × *glauca* is intermediate between the parental species for many morphological traits (e.g. Hotchkiss and Dozier 1945, Fassett and Calhoun 1952, Smith 1967, Grace and Harrison 1986, Waters and Shay 1990, Kuehn and White 1999, Olson et al. 2009) with the notable exception of height (it is often taller than either parental), and seed size/number (it often has fewer or smaller seeds; Marsh 1962).

The plant chemistry of *Typha* has not been well studied, but *T. angustifolia* appears to contain unidentified alkaloids and cyanogens not found in *T. latifolia* (Galatowitsch et al 1999), and *T. angustifolia* and *T. latifolia* differ qualitatively in the composition of soluble phenolics in root extracts (Jarchow and Cook 2009). Given that *Typha* species differ in some secondary compounds, and that most freshwater macrophytes including cattail are probably chemically defended (Prusak et al. 2005), it is plausible that some of the differences in arthropod abundance among *T. latifolia*, *T. angustifolia*, and *T.* × *glauca* are due to differences in herbivore-defense chemicals, or chemicals that affect arthropod preference for particular plants. *Typha latifolia* and *T. angustifolia* have been shown to differ in other aspects of leaf quality such as C:N ratio and lignin content (Maerz et al. 2010).

In addition to morphological and chemical differences, ecological differences among *Typha* species could influence arthropod abundance. For example, differences in habitat characteristics could influence the arthropod community that assembles on *Typha* plants by altering the pool of locally available arthropod species. *T. angustifolia* is more abundant than *T. latifolia* in deeper water, and tends to be displaced by *T. latifolia* in drier areas (Grace and Wetzel 1981a, 1998). *T. angustifolia* and *T.* × *glauca* both have a tendency to produce dense monocultures, though *T. angustifolia* reaches higher densities than the hybrid (Grace and Wetzel 1981a (TA/TL only), Larkin et al. 2011 (TG only), Waters and Shay 1992 (TG only)). *T.*

latifolia, on the other hand, often grows at lower densities as part of a more diverse plant assemblage (Grace and Wetzel 1981b, Grace and Harrison 1986). Some of the greater arthropod diversity we observed on *T. latifolia* is probably due to spillover from the communities on co-occurring plant species. *T.* × *glauca* produces a particularly enormous volume of litter, and alters its environment through shading (decreased water temperature), litter accumulation, and accumulation of large and different microbe communities that may help provide nitrogen that further fuels *T.* × *glauca* invasion (Angeloni et al. 2006, Farrer and Goldberg 2009, Vaccaro et al. 2009, Travis et al. 2010, Larkin et al. 2011). These ecological characteristics appear to contribute to *T.* × *glauca's* invasive tendencies, but they may also be related directly to the decreased abundance and diversity of arthropods on *T.* × *glauca* compared to *T. latifolia.*

It is likely that *Typha* genotypes also differ in their interactions with the decomposer community, either via direct effects of *Typha*, or via indirect effects mediated by differences in abundance of herbivorous insects. Herbivore presence, as well as plant genotype, has been shown in *Populus* to influence subsequent leaf decomposition dynamics, potentially altering ecosystem processes such as nutrient cycling (Schweitzer et al. 2005). Litter decomposition and detritus quality of wetland plants has also been shown to affect organisms such as tadpoles, either directly through the release of toxins, or indirectly by affecting the community of algae and other organisms upon which tadpoles feed (Maerz et al. 2010).

Our results have important implications for wetlands in areas of the United States where *T. angustifolia* is spreading and resulting in more frequent hybridization with *T. latifolia*. The hybrid is known for its vigorous growth and apparent tendency to outcompete both parental species, particularly *T. latifolia*, as well as other native wetland plants (Zedler and Kercher 2004). Our study shows that *T.* × *glauca* and *T. angustifolia* provide similar habitat quality for

arthropods in general, but the replacement of *T. latifolia* by *T.* × *glauca* would result in a marked decrease in local arthropod abundance. Although some species are more abundant on the hybrid than on *T. angustifolia*, it would be premature to conclude that *T.* × *glauca* marshes represent better arthropod habitats overall than *T. angustifolia* marshes. Certain species that are likely to play large ecological roles display depressed abundance on the hybrid (such as the important specialist herbivore *Limnaecia phragmitella*, and predators and parasitoids probably associated with it). In any case, *T. latifolia* vastly outperforms both *T. angustifolia* and the hybrid with regard to accumulating large and diverse arthropod communities. The effect of *Typha* genotype on the composition of functional communities such as decomposers is likely to be equally striking, and the implications for ecosystem function of cattail marshes make this an important area for future research.

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APPENDIX

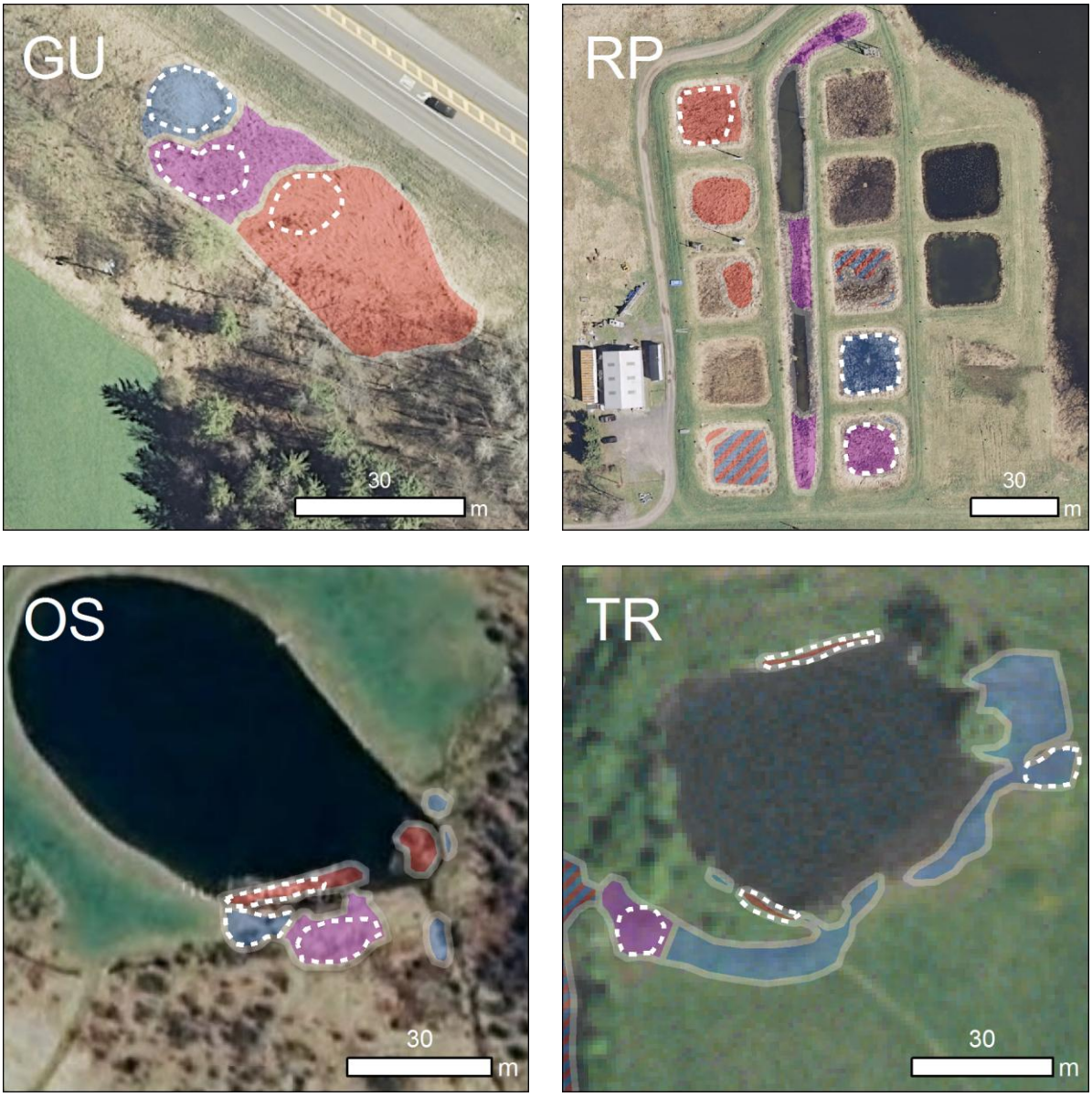


Figure 1.A1. Maps of the study sites showing the locations of each cattail species and the areas from which samples were taken. Blue is *T. latifolia*, red is *T. angustifolia*, and purple is *T.* × *glauca* . Areas with multiple species intermingled are hatched with the appropriate colors. Sampling areas are outlined with dotted lines. Aerial photos were obtained from USGS.

Figure 1.A1. (Continued)

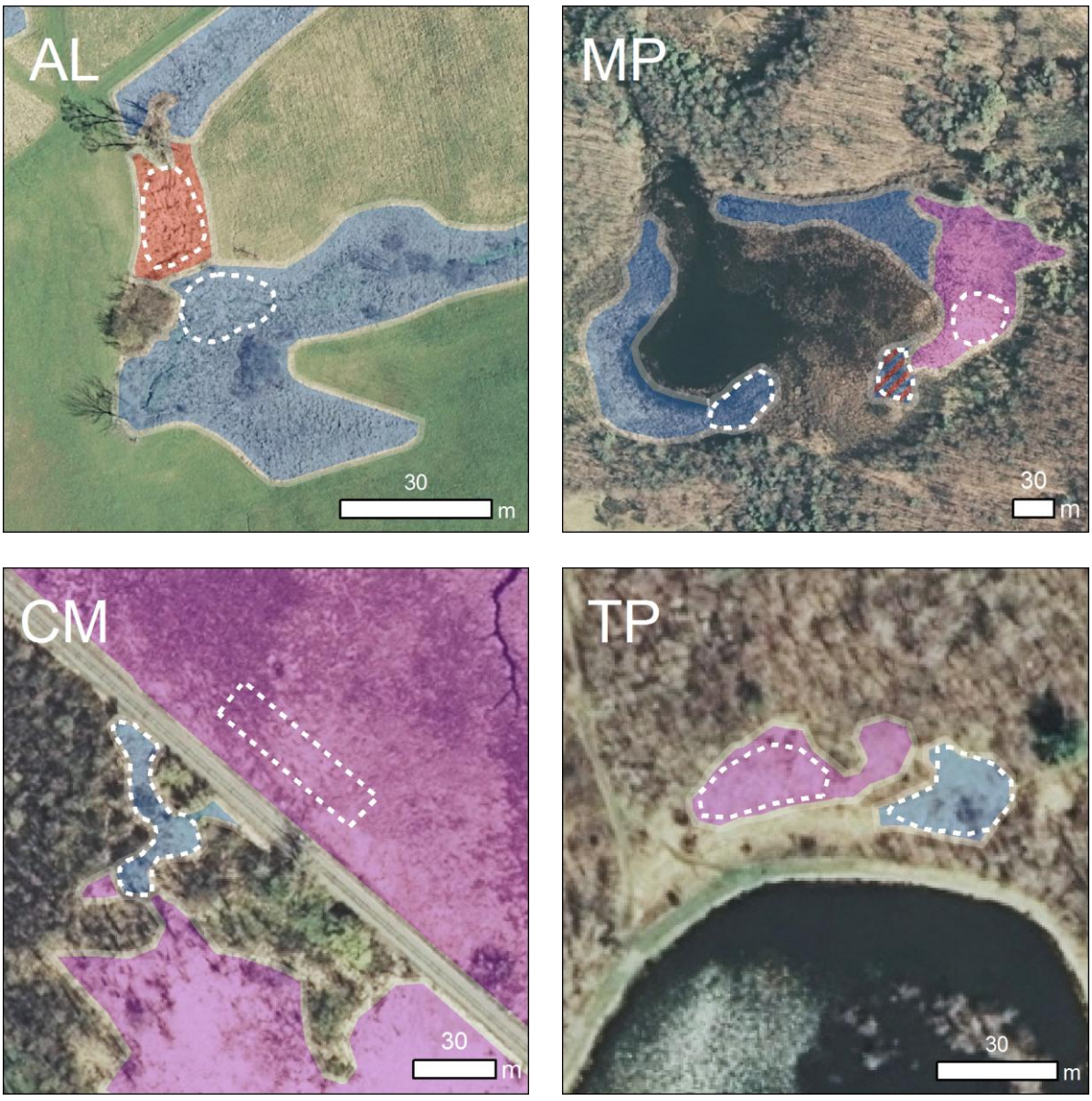
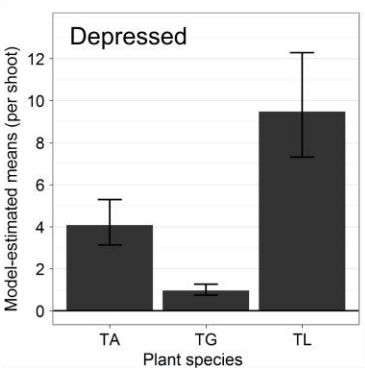


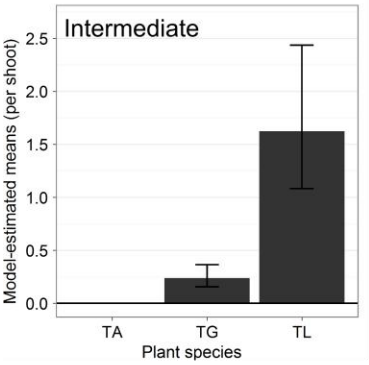
Figure 1.A2. Graphs of model-estimated mean abundance on *T. latifolia*, *T. × glauca* , and *T. angustifolia* for the species with sufficient sample size to include in the abundance pattern analysis. Bars represent +/- SE. Photographs of selected species are included.

Limnaecia phragmitella



7X

Dicymolomia julianalis

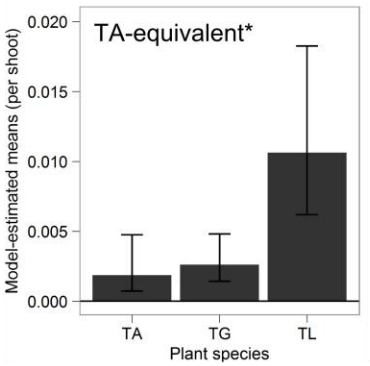


Rhynchophorus pertinax typhae

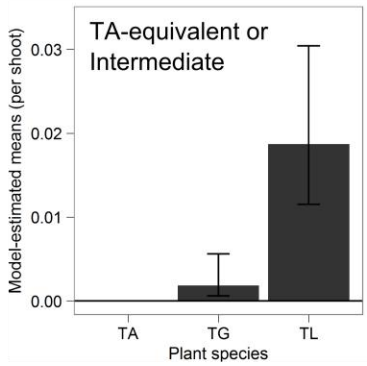
Only 2 found
(both on TL)

Figure 1.A2. (Continued)

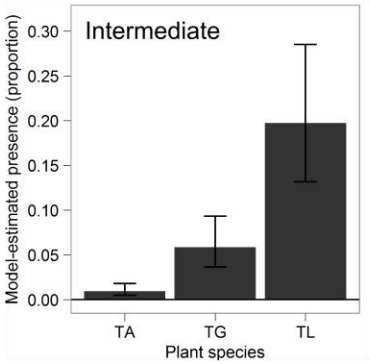
Bellura obliqua



Archanara oblonga



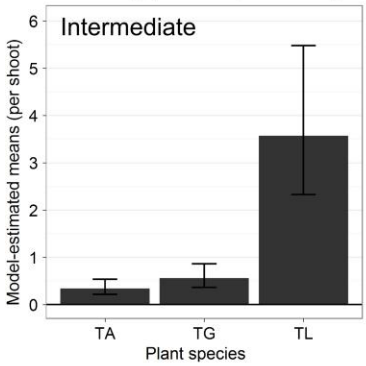
Bellura/Archanara burrows (binary)



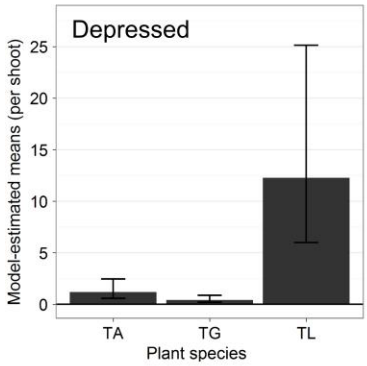
(burrow in cattail stem, containing larva; not to scale)

Figure 1.A2. (Continued)

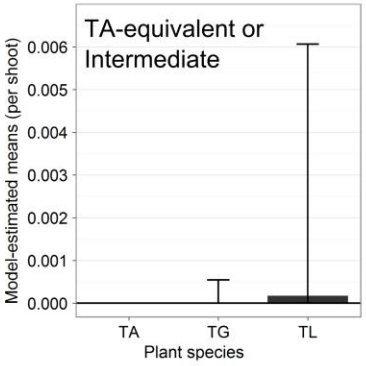
Chilacis typhae (adults)



Chilacis typhae (juvs)



Kleidocerys residae (adults)



Kleidocerys residae (juvs)

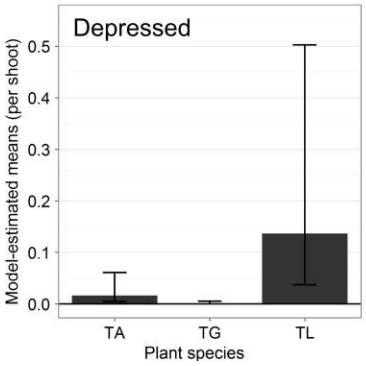
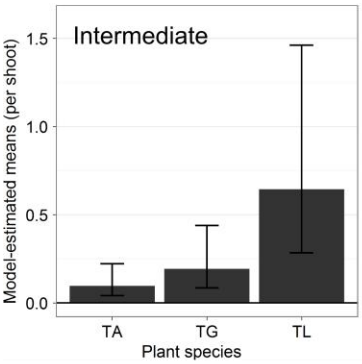
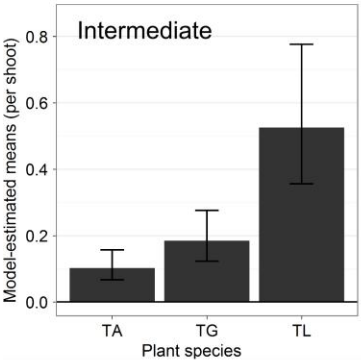


Figure 1.A2. (Continued)

Orthoperus sp.



Melanopthalma sp.



Sapintus fulvipes

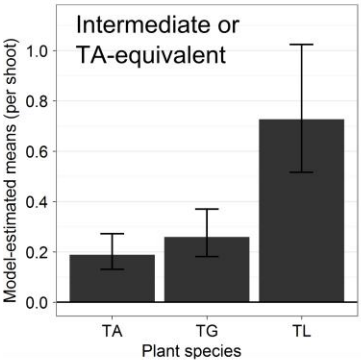
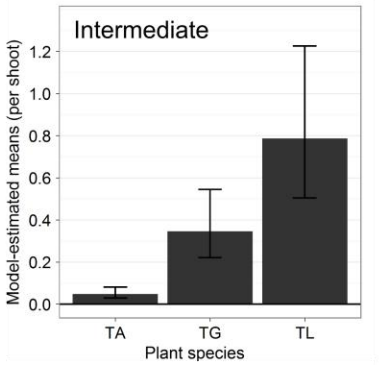
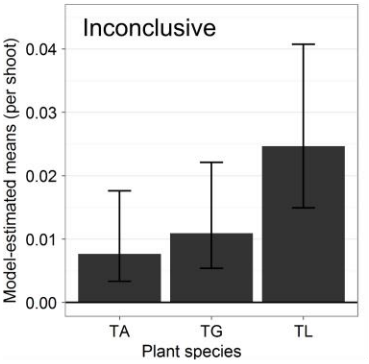


Figure 1.A2. (Continued)

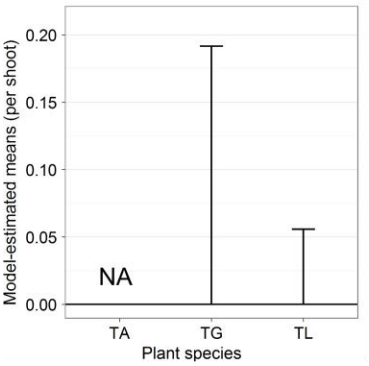
Sapintus lemniscatus



Phalacrid



Biphyllid



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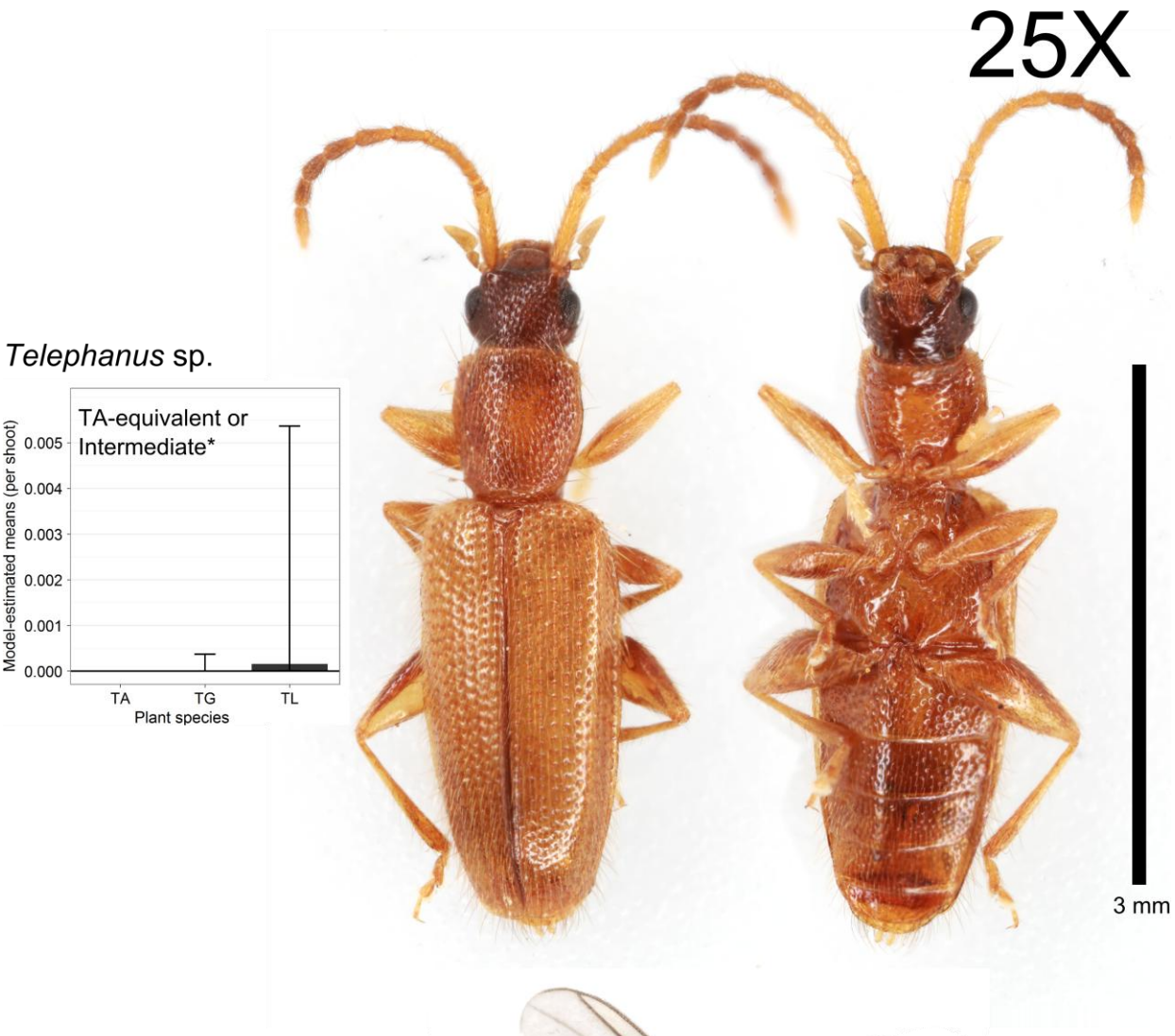


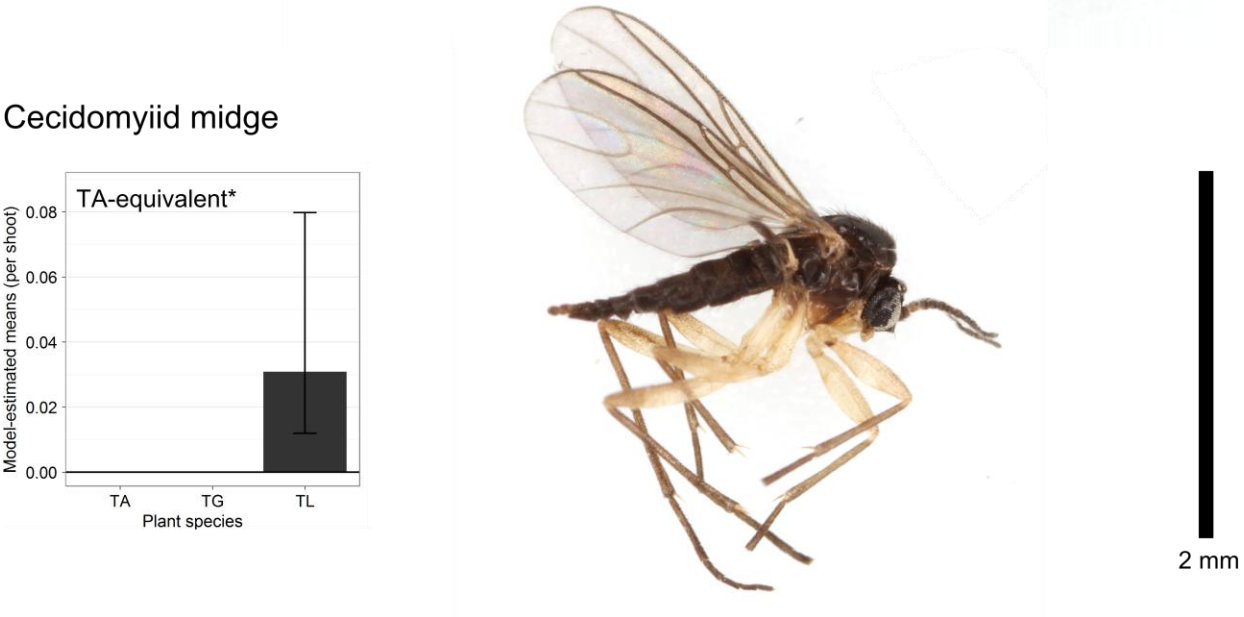
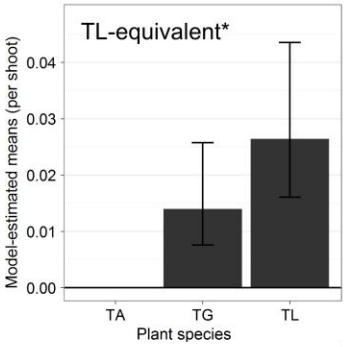
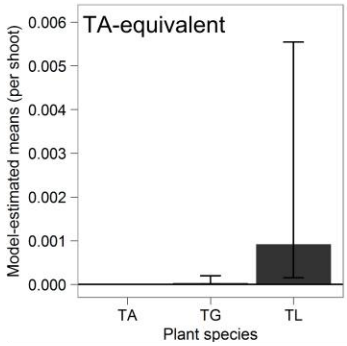


Figure 1.A2. (Continued)

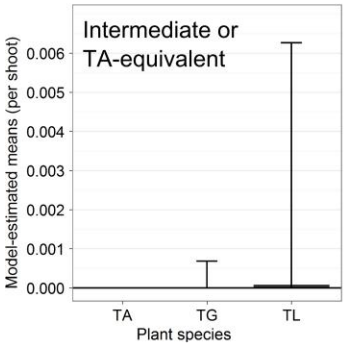
Stratiomyiid (adults from May survey)



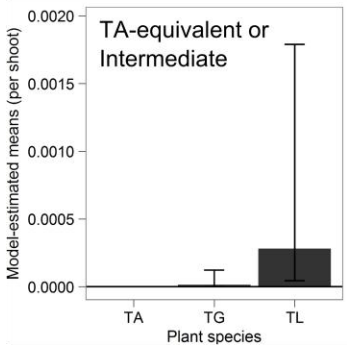
Stratiomyiid (larvae from July survey)



Eristalis (adults from May survey)



Eristalis (larvae from July survey)



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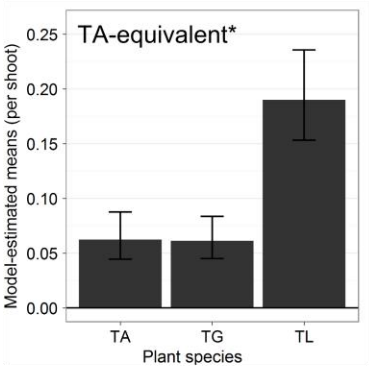
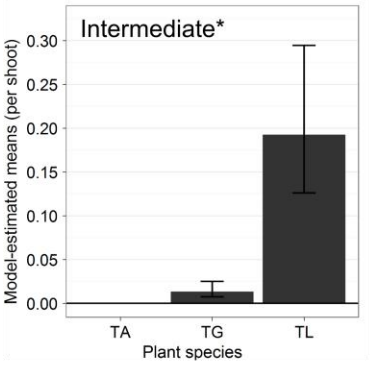
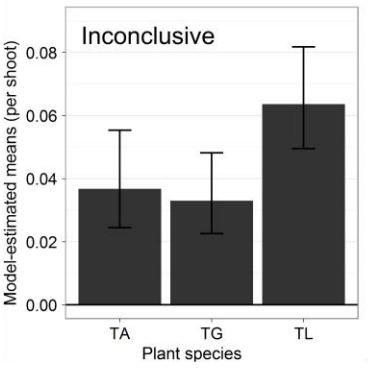
Hymenochaonia delicatus



Temelucha gracilipes



Scambus sp.



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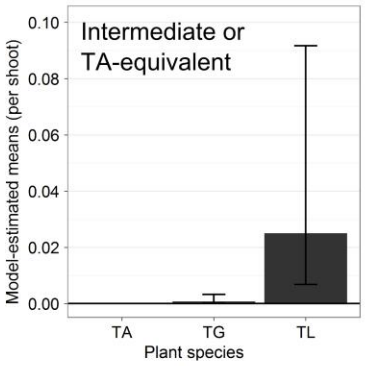
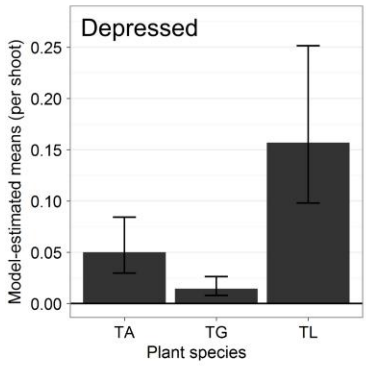
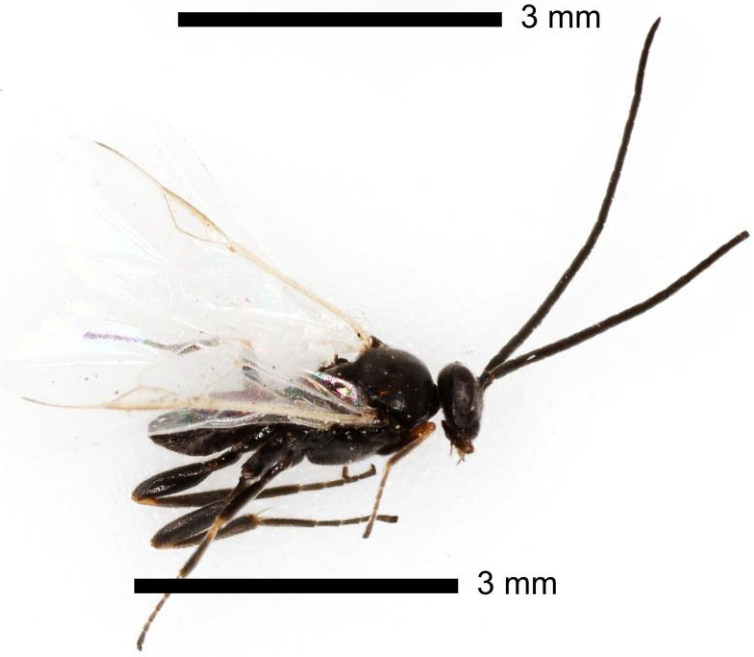
Chelonus sp.



Apanteles sp.





Macroteleia sp.

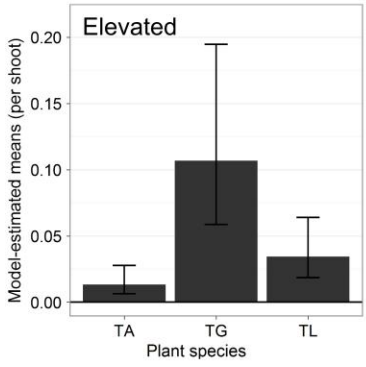
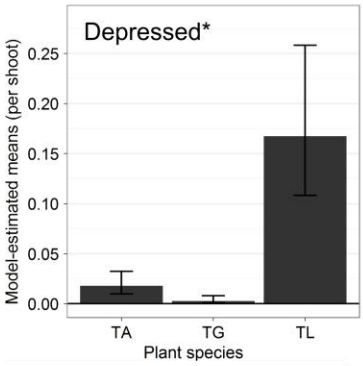
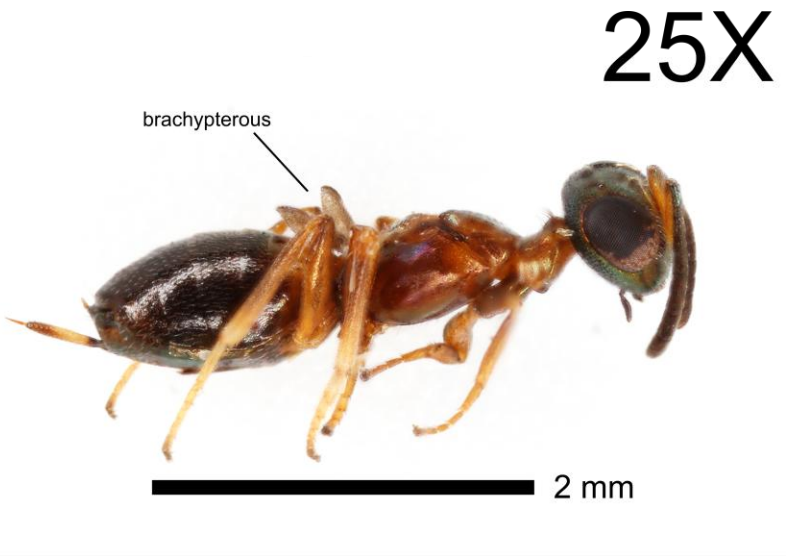


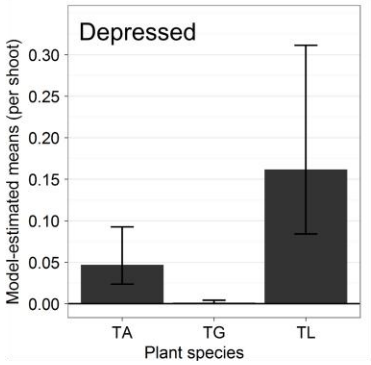
Figure 1.A2. (Continued)

Eupelmus dryorhizoxeni





Chalcedoid Q2



Chalcedoid Q1

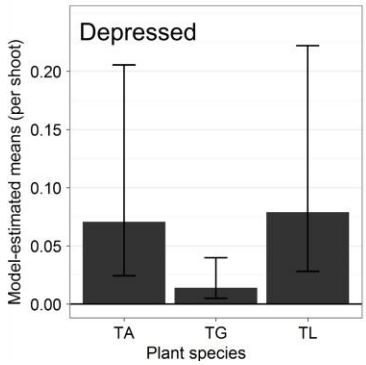
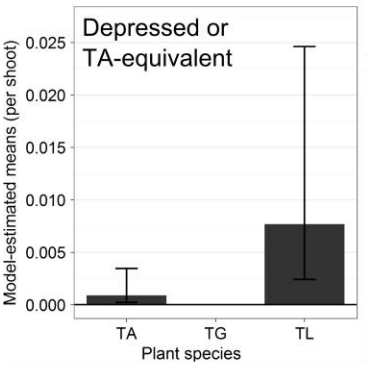


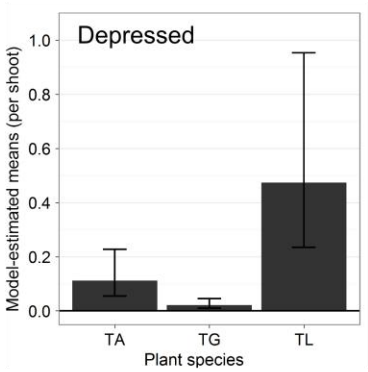
Figure 1.A2. (Continued)

25X

Chalcedoid Q7



Chalcedoid Q3



Chalcedoid Q4

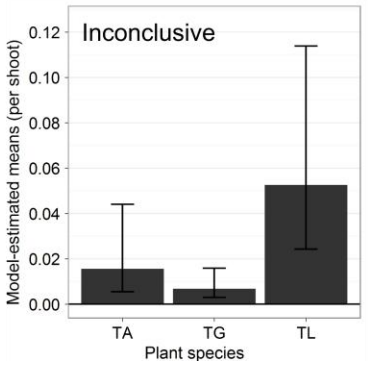
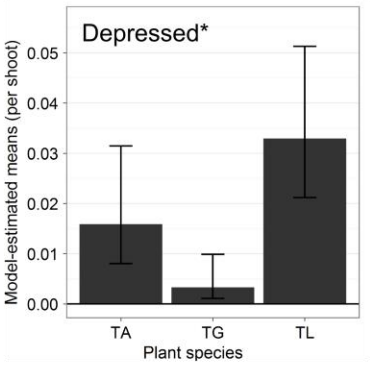
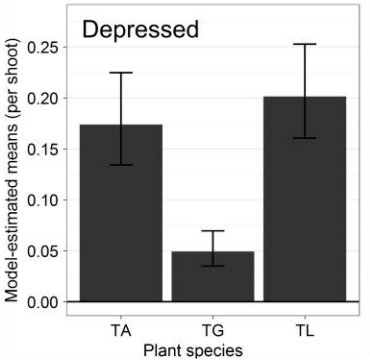


Figure 1.A2. (Continued)

Spider B1 (Clubionidae)



Sitticus sp.



Spider A2 (Araneidae)

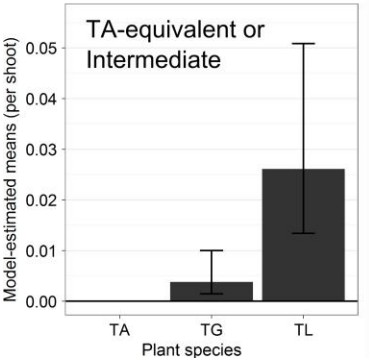
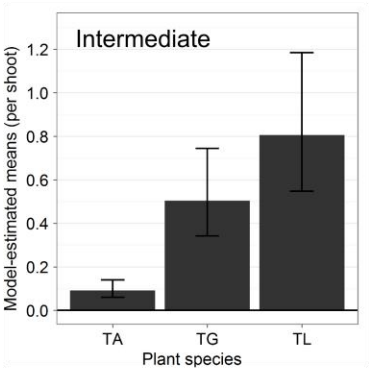


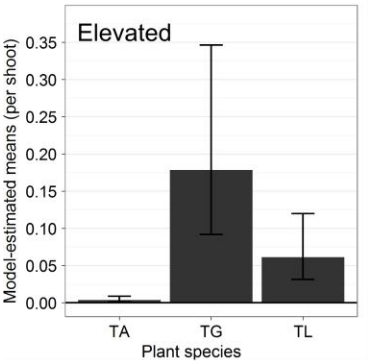
Figure 1.A2. (Continued)

50X

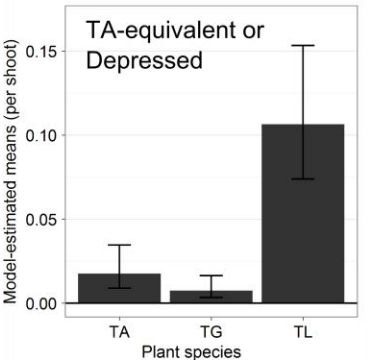
Thrips

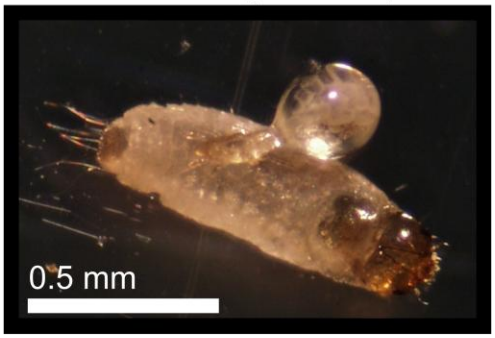


Mite (non-physogastric)



Physogastric mite (*Pyemotes* sp.)





Female mite feeding on 1st instar *L. phragmitella*

Figure 1.A2. (Continued)

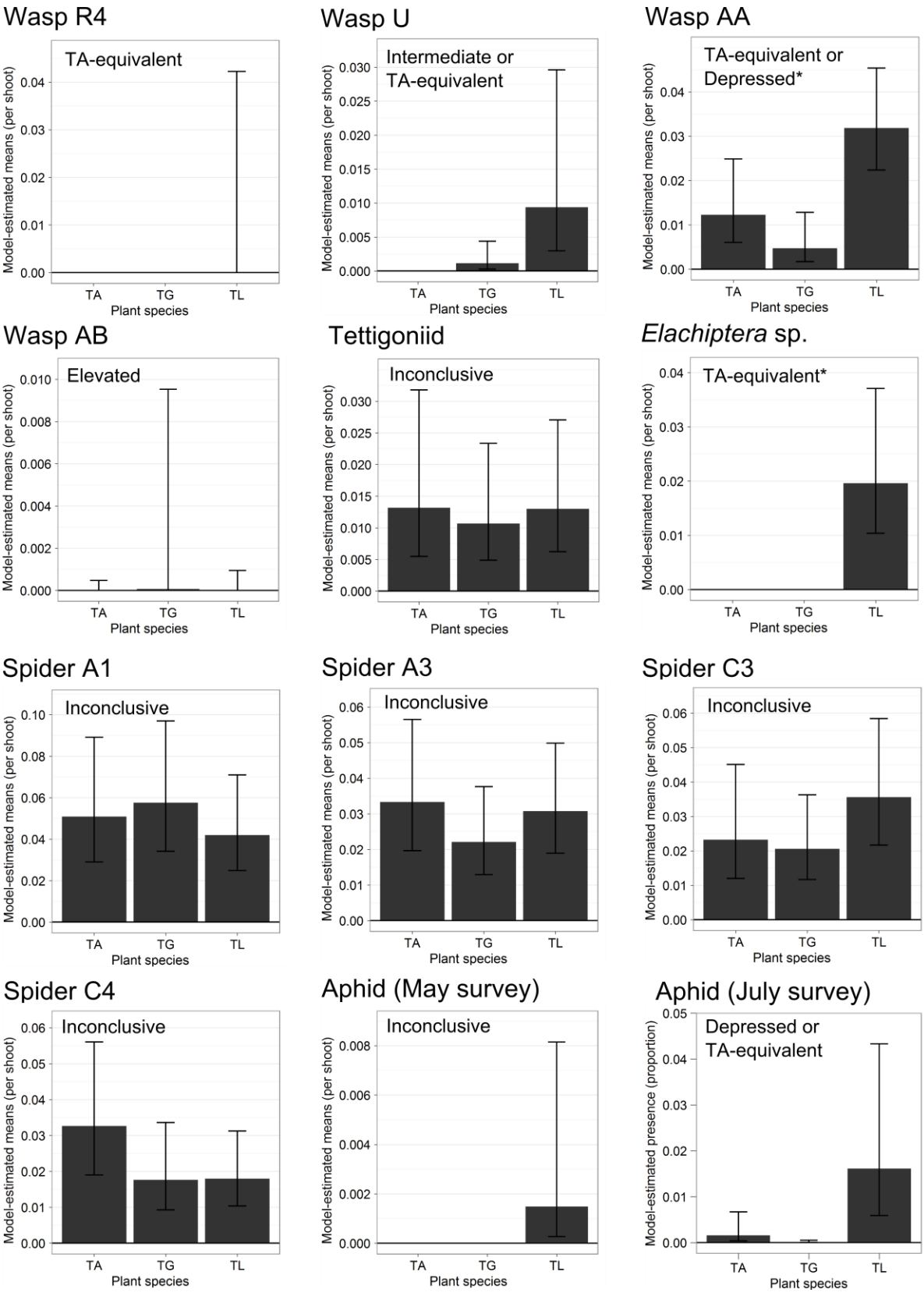
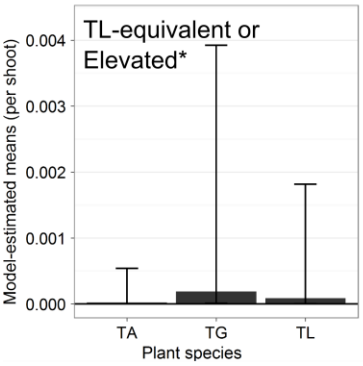
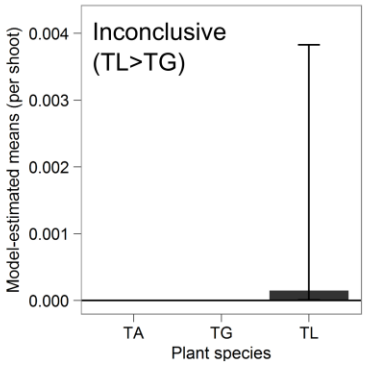


Figure 1.A2. (Continued)

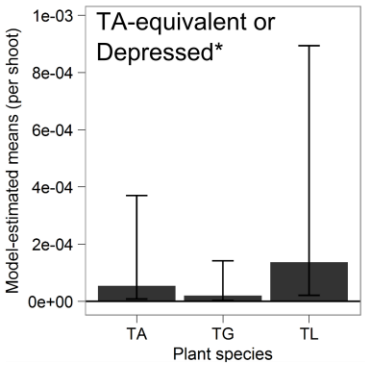
Psocid



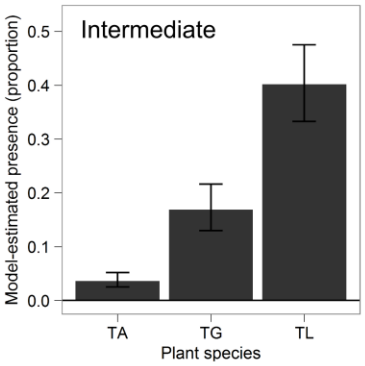
Scymnus sp.



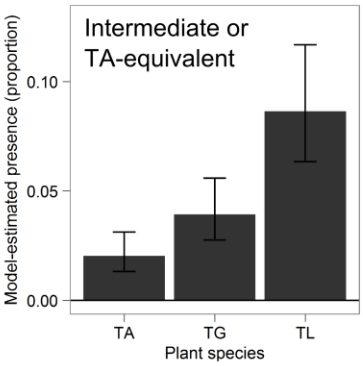
Ulidiid fly (unknown sp.)



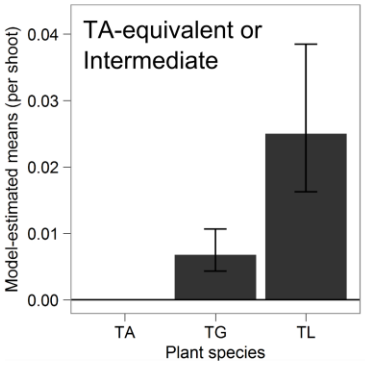
Herbivore damage (binary)



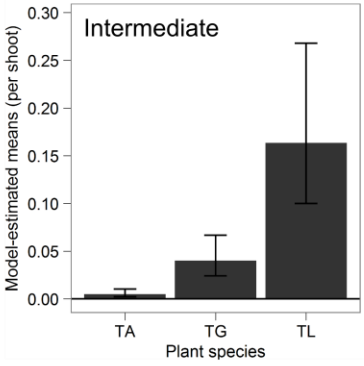
Central leaves dying (binary)



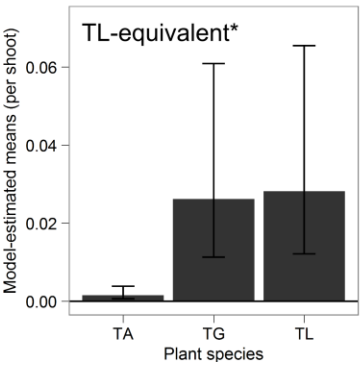
Chewed leaf centers (cm)



Bellura/Archanara stem holes



Chewed leaf edges (cm)



1st instar Archanara leaf cuts

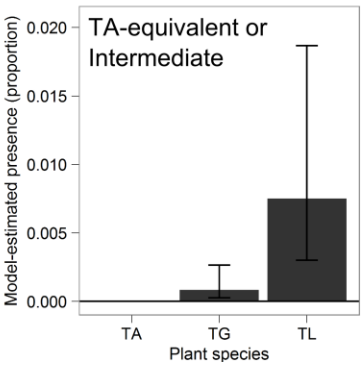


Table 1.A1. List of arthropods found in the May and July surveys, including the feeding mode used for the functional group analysis.

	Family or Superfamily	Survey	Feeding	Ecological Notes
Insecta				
Lepidoptera (7)				
Limnaecia phragmitella (emerged adults)	Cosmopterygidae	May	Herbivore	Seed feeder (*Typha* specialist)
Dicylomia julianalis (emerged adults)	Crambidae	May	Omnivore	Larvae feed on seeds and insects in the seed head
Archanara oblonga	Noctuidae	July	Herbivore	Stem borer
Bellura obliqua	Noctuidae	July	Herbivore	Stem borer
Bellura/Archanara burrows (binary)	Noctuidae	July	unassigned	Can't distinguish between burrows made by *Bellura* and *Archanara*
Adult microlep, unknown species	Unknown	May	unassigned	
Adult microlep, unknown species	Unknown	May	unassigned	
Adult microlep, unknown species	Tortricidae	May	unassigned	
Hemiptera (6)				
Chilacis typhae	Lygaeidae	May	Herbivore	Seed feeder (*Typha* specialist)
Kleidocerys resedae	Lygaeidae	May	Herbivore	Seed feeder
Assassin bugs (3 unknown species)	Reduviidae	May	Predator	Generalist insect predator
Frog hopper (unknown species)	Fulgoroidea	May	Herbivore	Phloem feeder
Aphids, unknown species (binary)	Aphidae	Both	Herbivore	Phloem feeder
Seed bug, unknown species	Lygaeidae	May	Herbivore	Seed feeder
Psocoptera (1)				
Bark louse, unknown species	Psocidae	May	Fungivore	Most feed on fungi and lichens on tree bark
Orthoptera (1)				
Long-horned grasshopper (unknown species)	Tettigoniidae	May	Herbivore	Emerged from stems. Eggs also found on stems but not counted.
Coleoptera (35)				
Orthoperus sp.	Corylophidae	May	Fungivore	Found in stems and heads
Melanopthalma sp.	Latridiidae	May	Fungivore	Found in stems and heads
Sapintus lemniscatus	Anthicidae	May	Fungivore	Found in stems only (*Typha* specialist?)
Sapintus fulvipes	Anthicidae	Both	Fungivore	Found in stems and heads

Table 1.A1. (Continued)

Phalacrid beetle (unknown species)	Phalacridae	May	Fungivore	Found in stems and heads
Telephanus sp.	Silvanidae	May	Detritivore	Usually is collected on ground, may be scavenger or predator
Biphyllid sp.	Biphyllidae	May	Fungivore	
Carabids (several unknown species)	Carabidae	Both	Predator	
Cantharid beetle (unknown species)	Cantharidae	May	Omnivore	Adults feed on pollen, nectar, or predaceous, larvae predaceous
Weevil (unknown species)	Curculionidae	May	Herbivore	
Rhynchophorus pertinax typhae	Curculionidae	July	Herbivore	Larva feeds in stem and rhizome (*Typha* specialist)
Flea weevil (Rhynchaeninae)	Curculionidae	May	Herbivore	Leaf feeder, larva is miner
Leguminous seed weevil (*Tychius* sp.)	Curculionidae	May	Herbivore	Seed feeder
Agasides hygrophila	Crysomelidae	May	Herbivore	Leaf feeder
Staphylinid (unknown species)	Staphylinidae	May	Predator	Most are predatory, some saprophytic
Staphylinid adult (possibly *Carpelimus*)	Staphylinidae	July	Predator	Most are predatory, some saprophytic
Ptillid adult (possibly *Acrotrichis*)	Ptillidae	Both	Fungivore	Found in rotting plant material
Bathona sp.	Corylophidae	May	Fungivore	Found in rotting plant material
Hydrophillid beetle (genus *Paracymus*?)	Hydrophillidae	May	Herbivore	Most adults are herbivores
Hydrophillid larvae (unknown species)	Hydrophillidae	July	Predator	Larvae feed on insects
Malporus sp.	Anthicidae	May	Fungivore	
Click beetle (unknown species)	Elateridae	May	Herbivore	Most larvae are root feeders, some predators
Dytiscid beetle (*Hydroporus* sp.)	Dytiscidae	May	Predator	
Toramus sp.	Languriidae	May	Fungivore	
Coleomegilla fuscilabris	Coccinellidae	May	Predator	Specialist on aphids
Helodid marsh beetle	Helodidae	July	unassigned	Larvae are aquatic filter-feeders, adults may not feed
Unknown species 1 (pieces only)	unknown	May	unassigned	
Unknown species 2 (pieces only)	unknown	May	unassigned	
Unknown species 3 (pieces only)	unknown	May	unassigned	
Unknown species 4 (pieces only)	unknown	May	unassigned	
Unknown species 5 (pieces only)	unknown	May	unassigned	
Unknown species 6 (pieces only)	unknown	May	unassigned	
Unknown species 7 (pieces only)	unknown	May	unassigned	
Unknown species 8 (pieces only)	unknown	May	unassigned	
Scymnus sp. larva	Coccinellidae	July	Predator	Prey on aphids, etc.

Table 1.A1. (Continued)

Diptera (11)				
Stratiomyid adults/larvae (unknown species)	Stratiomyidae	Both	Detritivore	
Eristalis sp. adults and larvae	Syrphidae	Both	Detritivore	Aquatic or semi-aquatic larvae. Adults feed on pollen and nectar.
Cecidomyiid adults	Cecidomyiidae	May	unassigned	Most are herbivorous, some are predators or parasitoids
Psychodid "moth fly" adult	Psychodidae	May	Detritivore	Larvae feed on fungi or algae in damp locations
Dolichopodid larva	Dolichopodidae	July	Predator	
Drosophilid larvae/pupae/adults	Drosophilidae	July	Detritivore	
Unknown fly species "S"		May	unassigned	
Elachiptera sp.	Chloropidae	May	Herbivore	Most are stem feeders
Unknown Ulidiid fly (larvae, pupae, adults)	Ottidae	Both	unassigned	Larvae are herbivores or detritivores
Unknown tiny fly		July	unassigned	
Sarcophagid (unknown species)	Sarcophagidae	July	unassigned	Scavengers, detritivores, or parasitoids
Hymenoptera (38)				
Ants (binary)	Formicidae	Both	Predator	
Bee	Apidae	May	Herbivore	
Ichneumonid cocoon	Ichneumonidae	May	Parasitoid	
Hymenochaonia delicatus	Braconidae	May	Parasitoid	Probably parasitoid of *L. phragmitella* and *D. julianalis*
Temelucha gracilipes	Ichneumonidae	May	Parasitoid	Probably parasitoid of *L. phragmitella* and *D. julianalis*
Scambus sp. ("D1")	Ichneumonidae	May	Parasitoid	Probably parasitoid of *L. phragmitella* and *D. julianalis*
Scambus sp. ("D2")	Ichneumonidae	May	Parasitoid	Probably parasitoid of *L. phragmitella* and *D. julianalis*
Wasp "E1"	Ichneumonidae	May	Parasitoid	
Wasp "E2"	Ichneumonidae	May	Parasitoid	
Wasp "E3"	Ichneumonidae	May	Parasitoid	
Macroteleia sp.	Scelionidae	May	Parasitoid	Egg parasitoid of Tettigoniids (long-horned grasshoppers)
Chelonus sp.	Braconidae	May	Parasitoid	Likely parasitoid of *L. phragmitella* and *D. julianalis*. Seed heads only.
Apanteles sp. ("I1")	Braconidae	May	Parasitoid	Likely parasitoid of *L. phragmitella* (not *D. julianalis*). Stems only.
Eupelmus dryorhizoxeni	Eupelmidae	May	Parasitoid	Reported as hyperparistoid of *Apanteles* (Gibson 2011).

Table 1.A1. (Continued)

Wasp L1 (Eurytominae)	Eurytomidae	May	unassigned	Lifestyle depends on genus: seed predators, secondary parsites, etc.
Wasp O (Diapriinae)	Diapreniidae	May	Parasitoid	Endoparasitism of various Diptera
Wasp Q1	Chalcidoidea	May	Parasitoid	Q1 and Q4 seem to occur together
Wasp Q2	Chalcidoidea	May	Parasitoid	Q2 and Q3 seem to occur together
Wasp Q3	Chalcidoidea	May	Parasitoid	
Wasp Q4	Chalcidoidea	May	Parasitoid	
Wasp Q5	Chalcidoidea	May	Parasitoid	
Wasp Q6	Chalcidoidea	May	Parasitoid	
Wasp Q7	Chalcidoidea	May	Parasitoid	
Wasp R1	Chalcidoidea	May	Parasitoid	
Wasp R4	Chalcidoidea	May	Parasitoid	
Wasp U	Unknown	May	Parasitoid	
Wasp V	Chalcidoidea	May	Parasitoid	
Wasp X	Unknown	May	Parasitoid	
Wasp Z	Chalcidoidea	May	Parasitoid	
Wasp AA	Unknown	May	Parasitoid	
Wasp AB	Unknown	May	Parasitoid	
Wasp AC	Ichneumonidae	May	Parasitoid	
Wasp AD	Chalcidoidea	May	Parasitoid	
Wasp AE	Chalcidoidea	May	Parasitoid	
Wasp AF	Unknown	May	Parasitoid	
Wasp AG	Chalcidoidea	May	Parasitoid	
Wasp AH	Ichneumonidae?	July	Parasitoid	Parasitoid of one or both Noctuid borers (*Bellura and Archanara*)
Wasp AJ	Unknown	July	Parasitoid	
Thysanoptera (2)				
thrips (single species)	Unknown	May	Herbivore	Phloem feeder
thrips (other)	Unknown	May	Herbivore	Phloem feeder
Collembola (1)				
misc. springtails	Unknown	May	unassigned	

Table 1.A1. (Continued)

Arachnida				
Araneae (20)				
Emblyna hentzi (Spider A1)	Dictynidae	May	Predator	Web spinners; probably had web between two cattail plants
Spider A2	Araneidae?	May	Predator	Probably a web spinner
Araneus sp. (Spider A3)	Araneidae	May	Predator	Web spinners; probably had web between two cattail plants
Araneus sp (Spider A4)	Araneidae	May	Predator	Web spinners; probably had web between two cattail plants
Spider A5	Araneidae?	May	Predator	Very similar to *Emblyna hentzi*
Spider B1	Clubionidae	May	Predator	Active hunters
Spider B2	Clubionidae	May	Predator	Active hunters
Spider B3	Clubionidae	May	Predator	Active hunters
Sitticus sp., prob. *S. floricola palustris*	Salticidae	May	Predator	Active hunters. Reside in fluff of senesced seed head.
Spider C3	Salticidae	May	Predator	Active hunters. Reside in fluff.
Spider C4	Salticidae	May	Predator	Active hunters. Reside in fluff.
Spider C5	Salticidae	May	Predator	Active hunters
Spider C6	Salticidae	May	Predator	Active hunters. Reside in fluff.
Spider C7	Salticidae	May	Predator	Active hunters
Spider C8	Salticidae	May	Predator	Active hunters
Spider C9	Salticidae	May	Predator	Active hunters
Spider E	Tetragnathidae	May	Predator	Web spinners; probably had web between two cattail plants
Spider E2	Tetragnathidae	May	Predator	Web spinners; probably had web between two cattail plants
Spider F	Thomisidae	May	Predator	Active hunters or ambushers
Micaria sp. (Spider G)	Gnaphosidae	May	Predator	Active hunters.
Acari (3)				
Physogastric mite (number of shoots with)	Pyemotidae	May	Parasite	Parasitic on eggs, larvae, pupae of *L. phragmitella* and *D. julianalis*. Probably phoretic.
Mite (unknown species)	Unknown	May	unassigned	Unknown
Other mites (multiple species)	Unknown	May	unassigned	Unknown
Crustacea				
Terrestrial isopod (Isopoda) (1)	Unknown	May	Detritivore	
Myriopoda				
Diplopoda (1)	Unknown	July	Detritivore	

CHAPTER 2

TROPHIC ANALYSIS OF TWO LEPIDOPTERAN SPECIES (*LIMNAECIA PHRAGMITELLA* AND *DICYMOLOMIA JULIANALIS*) COMMON ON *TYPHA* SPP.

ABSTRACT

Cattail plants are ecologically and economically important wetland plants in the United States. The biology of the insect communities associated with cattail plants is of interest to researchers concerned with wetland habitat quality, or to those using *Typha* as a model system for ecological and evolutionary questions. We used nitrogen isotope analysis to clarify the trophic relationships between *Typha* and two Lepidopteran species reported to feed on cattail and touted in the literature as ecologically equivalent seed-feeding herbivores. We show that while the specialist moth *Limnaecia phragmitella* appears to be an herbivore, the second moth *Dicymolomia julianalis* displays more enriched isotope signatures consistent with an omnivorous diet. This conclusion is consistent with other research on *D. julianalis* describing it as a possible scavenger, and our own observations of predatory behavior in these larvae. Methodologically, our study also provides an interesting example of spatial variation in isotope signature on an unusually small scale.

INTRODUCTION

Cattail plants are widespread and abundant in the United States and represent an ecologically dominant wetland plant species, as well as an important invader of disturbed wetlands in many regions. Cattails support a diverse arthropod assemblage, and the fluff of the senesced seed-heads, which frequently persists intact through the winter, hosts a rich arthropod community of seed feeders, fungus feeders, parasitoids and predators (Claassen 1921, see Chapter 1).

Two of the most commonly found arthropods on cattail are larvae of the moths *Limnaecia phragmitella*[1] (Cosmopterigidae) and *Dicymolomia julianalis* (Crambidae[2]). Both species have been used as examples in studies of herbivore response to interspecific hybridization of their host plants (Eisenbach 1996, Fritz 1999, Fritz et al. 1999, Whitham et al. 1999) despite the fact that the natural history of *D. julianalis* is not well understood. Although it is supposedly a seed-feeder on cattail plants (Claassen 1921), it has also been recorded from seed heads of musk thistle *Carduus nutans* (Powell et al. 1992), Le Conte's thistle *Cirsium lecontei* and milk vetch *Astragalus canadensis* (Munroe 1972), cotton bolls *Gossypium* sp. (Heinrich 1921), prickly pear joints *Opuntia* sp. (Hunter et al. 1912), and perhaps more strangely, the cases of bagworm moths *Thyridopteryx ephermeraeformis* (Gahan 1909, McCreary 1930, Balduf 1937, Barrows and Gardh 1974, Kaufmann 1985, Landau 1996), where it is reported to feed on

[1]This species was originally described as *Limnaecia phragmitella* by Stainton (1851). He later moved it to the genus *Laverna* (Stainton 1858). It was subsequently moved back to *Limnaecia* by Meyrick (1888). Dyar et al. (1902) misspelled the genus as *Lymnaecia* in his checklist. The mistake was perpetuated by Claassen (1921), Eisenbach (1996), and others.

[2]Many papers continue to refer to this species as a Pyralid. The subfamily Crambinae was separated from Pyralidae and elevated to family status (Crambidae) by Minet (1983) based on tympanal organs, and he also moved many subfamilies of Pyralidae into Crambidae, including the Glaphyriinae, which includes *Dicymolomia*. Monroe and Solis (1998) confirmed that the rearrangement was warranted.

the eggs (Gahan 1909, McCreary 1930, Landau 1996). Kaufmann (1985) reports finding *D. julianalis* larvae inside bagworm larvae and pupae, with individuals that had consumed eggs showing a higher emergence rate. *Thyridopteryx ephermeraeformis* is found on a variety of tree and shrub species, particularly red cedar (*Juniperus virginiana*), and *D. julianalis* has been found in bagworm cases on multiple plant species. Given the diversity of its habits, it is reasonable to ask whether *D. julianalis* represents several cryptic species or perhaps a collection of genetically differentiated host races. McCaskill (1995) used allozymes to investigate host race differentiation of *D. julianalis* on musk thistle, cattail, and bagworms, and found some evidence of differentiation among those groups. In a phenological analysis, populations from across Tennessee grouped by host species rather than location, except for one musk thistle population that grouped with bagworm. Musk thistle and bagworm populations were more similar to each other than to cattail populations. However, this study was never published except as a master's thesis, and no further work has addressed the question of host race evolution in this species.

The natural history of *L. phragmitella* is less controversial. A specialist on *Typha* spp., the larva has been described as feeding on developing cattail flowers and then seeds (Claassen 1921, Eisenbach 1996). However, many Lepidoptera will consume other insects (including conspecifics) opportunistically, or under certain conditions (e.g. Ware & Stephen 2006, Pierce 1995). It is not known whether *L. phragmitella* is strictly herbivorous or whether omnivorous feeding habits complicate its ecological role and relationship to *Typha*. This question is of particular interest to researchers studying the response of insects to hybrid host plants, since *L. phragmitella* has been presented in the literature as a good example of hybrid resistance, a phenomenon in which insects are less abundant on a hybrid plant than on either of the parental species (Fritz et al. 1994, Fritz 1999, Whitham et al. 1999). There is evidence that *T.* × *glauca*

represents a poor quality host to *L. phragmitella* larvae because of low seed abundance (See Chapter 3). Knowing whether *L. phragmitella* is able to shift its diet when faced with poor quality host plants is important to understanding what drives the differences in *L. phragmitella* abundance on hybrid and parental cattails. In this study, we used stable isotope analysis to unravel the trophic relationships of *D. julianalis* and *L. phragmitella,* and determine whether they can legitimately be called herbivores feeding on cattail.

METHODS

We obtained nitrogen isotope signatures ($\delta^{15}N$) for *L. phragmitella* (N=62) and *D. julianalis* (N= 37) individuals collected from the three cattail species (*T. latifolia*, *T. angustifolia*, and the hybrid *T. x glauca*) growing at a single site (Mud Pond, McLean, NY[3], Figure 2.1). *Dicymolomia julianalis* were not collected from *T. angustifolia* because they do not occur on that host plant (Eisenbach 1996; see also Chapter 1). Insects were sampled from *T. latifolia* growing south of the pond (area A) as well as from *T. latifolia* growing intermingled with *T. angustifolia* about 100 m to the east (area B). Moths were reared from senesced shoots containing larvae/pupae, so their host origin is known. Upon emergence, moths were frozen until testing.

Tissue from the exact host plants was not available to obtain baseline $\delta^{15}N$ values, since insects were collected from senesced shoots and the fluff of the old seed-heads was contaminated with frass, fungus, and insect parts. Instead, we used green leaf tissue from shoots growing in the same location as the shoots from which the insects were previously collected. Since the insects supposedly eat the flowers and seeds, not the leaves, we performed a pilot study to determine whether leaf tissue differed in isotope signature from seed-head tissue. For a subset of shoots, we tested samples of homogenized cattail "fluff" containing seeds and flower parts from mature

[3] This is the same site as Claassen (1921) used for his early studies of cattail insect communities.

cattail heads. All plant samples were collected in the summer following the collection of moths from senesced shoots of the previous year, and plant material was frozen until testing.

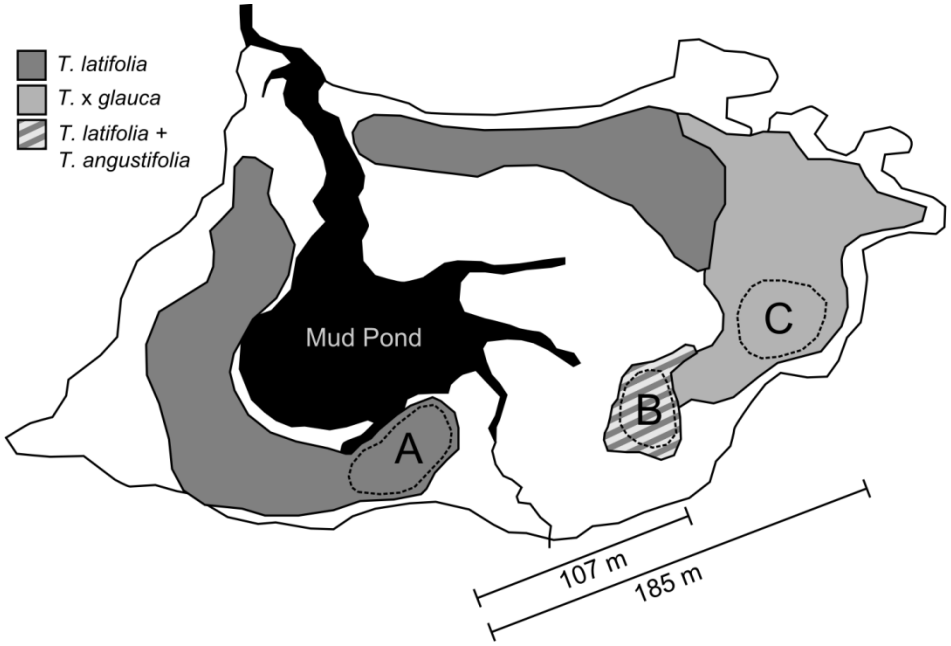


Figure 2.1. Map of Mud Pond, near McLean, NY, where cattail plant and moth samples were obtained for isotope analysis. The shading indicates the location of the three cattail species at the site. The circled areas labeled A, B, and C mark the exact locations from which plants were sampled. *T. latifolia* plants were sampled both from area A, and from area B where they grew intermixed with *T. angustifolia*.

For isotope analysis, plant leaf tissue was freeze-dried and ground using a Genogrinder. Seed-head tissue was ground under liquid nitrogen with a mortar and pestle. Whole insects were dried in a drying oven and homogenized by crushing with a small metal spatula. Individual insects were analyzed separately. For plants, about 3 mg ground tissue was used for analysis; for insects, about 1 mg was used (this was often, but not always, all the recoverable tissue after homogenization). Isotope analysis was performed by the Cornell Stable Isotope Laboratory (COIL) using a Thermo Delta V isotope ratio mass spectrometer (IRMS) interfaced to an NC2500 elemental analyzer.

For the pilot study comparing leaf tissue to seed-head tissue, a linear regression using the lm() function in R (R Development Core Team 2012) was performed to estimate the slope of the

relationship between seed head and leaf $\delta^{15}N$ values. For the principle analysis, isotope values were analyzed with ANOVA using the aov() function, with isotope value as a function of trophic group (host plant, *L. phragmitella*, or *D. julianalis*) and sampling group, which is a combination variable including *Typha* species (*T. latifolia*, *T. angustifolia*, or *T. x glauca*) and area of the marsh (A, B, or C; see map, Figure 2.1). Contrasts were performed using the glht() function in the multcomp package (Hothorn et al 2008), and p-values were adjusted for multiple comparisons using Tukey's method.

RESULTS

Leaf tissue and seed-head tissue were shown to be very similar in isotope signature. The estimate for the slope of the relationship was 1.12, which is very close to the expected slope of 1 if the isotope values for the two tissue types are actually equal (See Figure 2.2). Since the values for the leaf tissue and seed head tissue were comparable, we used leaf tissue for all further analyses because there were a number of plants for which fluff was unavailable.

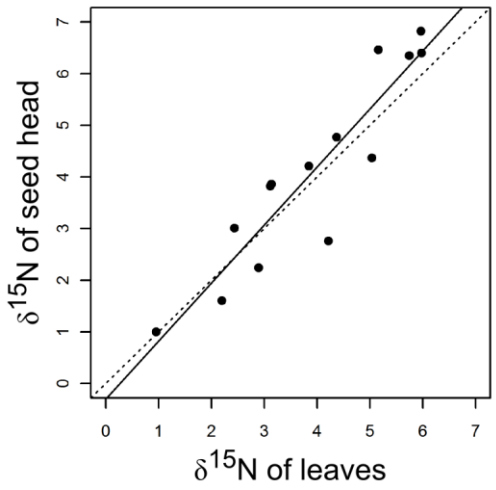


Figure 2.2. Nitrogen $\delta^{15}N$ isotope values of leaves vs. seed head tissue for 14 plants from Mud Pond, McLean, NY. The solid black line is the regression line from the linear model, with an estimated slope of 1.1. The dotted line (plotted as a reference) has a slope of 1.

Estimated mean isotope signatures for the *Typha* species showed highly significant differences among sampling groups (for all significant contrasts, $p<0.001$), with *T.* × *glauca* (area C) having higher $\delta^{15}N$ values than *T. angustifolia* (area B) and *T. latifolia* (area A and B). *Typha angustifolia* had significantly higher values than *T. latifolia* in area A, but there was no significant difference between *T. angustifolia* and *T. latifolia* growing in the same area (area B; $p=0.805$). *Typha latifolia* in area B had significantly higher values than the same species in area A.

Trophic analysis relies on differences in isotope ratios between consumers and their food sources. Despite the differences in the plant isotope values, the interaction between sampling group and trophic group was not significant ($p=0.599$). In other words, the differences in isotope signatures between the insect species and their host plants were consistent across all cattail sampling groups (e.g., insects from *T.* × *glauca* in area C were correspondingly more enriched than insects from *T. latifolia* in area A; see Figure 2.3). Estimates for differences in isotope signatures showed that *L. phragmitella* differed from the plants by 3.4 per mil (95% CI: 2.8-4.1‰), whereas *D. julianalis* differed from the plants by 5.6 per mil (95% CI: 4.8-6.4‰). *D. julianalis* differed from *L. phragmitella* by 2.2 per mil (95% CI: 1.5-2.9‰).

For a subset of plants (n=25), both *L. phragmitella* and *D. julianalis* individuals were available, enabling comparison of *L. phragmitella* and *D. julianalis* isotope values independent of plant baseline, which is useful in this case because isotope values for the exact host plants of the moths in the study were unavailable (see Methods). The mean paired difference for *L. phragmitella* and *D. julianalis* from the same host plants was 2.2 (Figure 2.4), which is consistent with the value obtained above using the plant baselines.

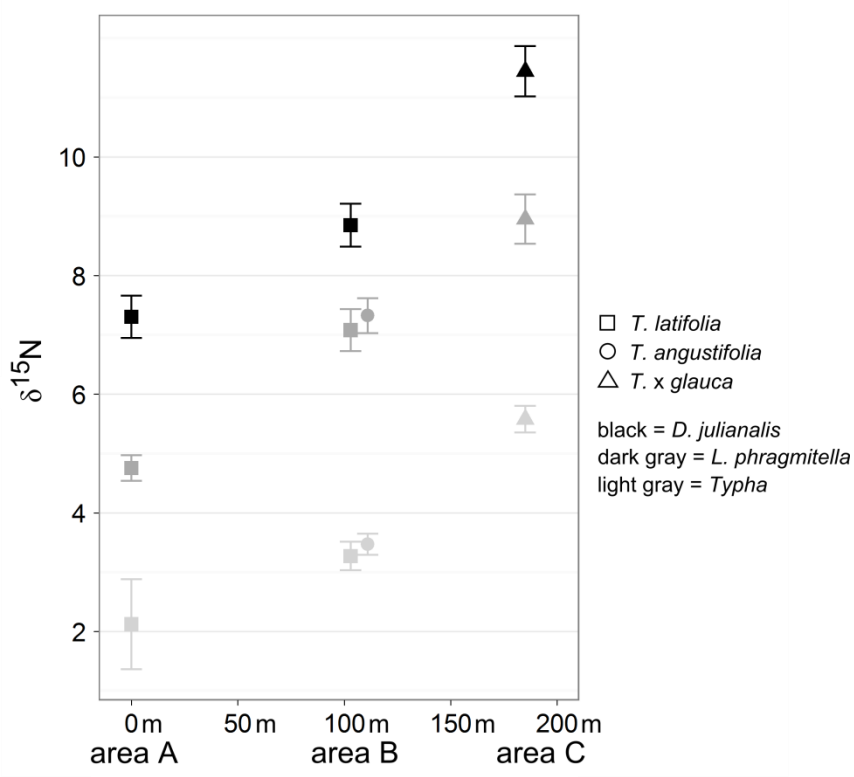


Figure 2.3. Model-estimated mean nitrogen isotope values ($\delta^{15}N$) for *L. phragmitella*, *D. julianalis*, and their host plants, *T. latifolia*, *T. angustifolia*, and *T.* × *glauca* at from areas A (0 meters), B (107 m) and C (185 m) at Mud Pond. Distances are relative to the center of area A. Area B has both *T. latifolia* and *T. angustifolia* plants. Error bars represent ± 1 SE.

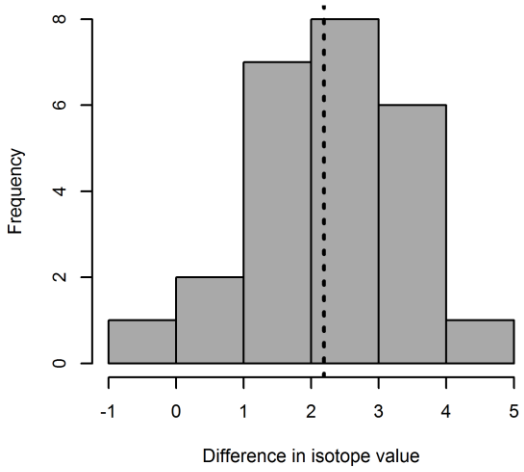


Figure 2.4. Differences in $\delta^{15}N$ between *L. phragmitella* and *D. julianalis* pairs from the same plant. The dotted line is the mean value (2.2).

DISCUSSION

For nitrogen, the stable isotope ratios ($\delta^{15}N$) of consumers are typically enriched by approximately 3 per mil compared to their food source (e.g. DeNiro and Epstein 1981), and Post (2002) calculated a mean value of 3.4‰ (normally distributed with standard deviation 0.98) across a range of published studies. Based on these estimates, our results support the characterization of *L. phragmitella* as an herbivore (estimated difference between *L. phragmitella* and *Typha* is 3.4‰). There was no evidence that *L. phragmitella* predictably shifts its diet on different species of *Typha*, as the relationship was consistent on all three cattail species.

The $\delta^{15}N$ values for *D. julianalis* relative to the *Typha* baseline were considerably higher (estimated difference 5.6 ‰). This difference strongly suggests that *D. julianalis* is not feeding entirely on *Typha*, but is incorporating other food sources into its diet and cannot legitimately be called an herbivore. We have observed that *D. julianalis* readily attacks and consumes larvae of *L. phragmitella* (see Figure 2.5), confirming its status as a likely predator in cattail heads, and suggesting a possible source of the over-enriched $\delta^{15}N$ values with respect to the plants. In fact, it is not really known whether *D. julianalis* eats *Typha* (seeds and/or other tissue) at all. Since our 95% confidence interval around the $\delta^{15}N$ estimate for *D. julianalis* ranges up to 6.4‰, it is possible that *D. julianalis* is (at least in some cases) a scavenger/predator with no direct contribution from *Typha* to its diet.

Figure 2.5. Stills from a video of a large *D. julianalis* larva attacking and consuming a first-instar *L. phragmitella* larva.

Claassen (1921) characterized *D. julianalis* as a seed-feeding herbivore on *Typha*, and was apparently unaware of several earlier references to this species' diverse feeding habits and characterization as a scavenger (Gahan 1909a, Gahan 1909b, Quaintance 1909, Dyar 1909, Hunter et al. 1912). Claassen's characterization may be in error because he seems to have confused eggs of *L. phragmitella* with those of *D. julianalis*[4], and the first instar larvae he observed hatching and feeding were actually *L. phragmitella*. It is not clear whether he ever observed actual *D. julianalis* feeding on *Typha*. Eisenbach (1996) seems to have relied entirely on Claassen (1921) for his natural history information about *D. julianalis*, despite the existence of a substantial body of literature regarding *D. julianalis* (Heinrich 1921, McCreary 1930, Balduf 1937, Munroe 1972, Barrows and Gardh 1974, Allyson 1981, Covell 1984, Kaufmann 1985, Powell et al. 1992), in which diverse feeding habits are discussed. Subsequent inclusion of *D. julianalis* in meta-analyses of herbivore abundance patterns on hybrid plants (Whitham et al. 1999, Fritz 1999) was based upon Eisenbach (1996), although several more papers discussing *D. julianalis* feeding habits had since been published (Landau et al. 1996, Powell et al. 1996, Solis and Adamski 1998).

[4] The eggs of *D. julianalis* are described by Kaufmann (1985), whose description matches our own observations. The eggs described by Claassen (1921) match our observations of *L. phragmitella* eggs.

Our findings for *D. julianalis* on cattail may also be suggestive of its role in the seed heads of musk thistle, as it is not known whether its diet is restricted to seeds or if it preys on other insects in that system (Powell et al. 1992). Given our findings in cattail seed-heads, it seems unlikely that *D. julianalis* is ever an exclusive herbivore. However, whether the larvae obtain animal tissue primarily through scavenging or through predation is not known, and may vary depending upon circumstances. *Dicymolomia julianalis* has been suspected of scavenging in several systems including cotton (Heinrich 1921), bagworm cases (Balduf 1937), and thistle (Landau et al 1996). The only system where it has been previously shown to be predatory is in the bagworm case (Gahan 1909, Kaufmann 1985, Landau et al. 1996), although *D. julianalis* larvae can themselves be consumed by the bagworm larvae once these hatch (Kaufmann 1985, Landau et al. 1996).

Landau et al. (1996) noted that competition for food or cannibalism might explain why they found only one *D. julianalis* emerging from the vast majority of musk thistle seed heads and bagworm cases. Kaufmann (1985) observed that in bagworm cases containing two *D. julianalis* larvae, the first to pupate would often be consumed by the other larva. They observed that two larvae in close proximity would often attack each other, a behavior that we also observed in *D. julianalis* from cattail. In cattail, however, many *D. julianalis* adults frequently emerge from the same seed head. It remains a mystery why *D. julianalis* is not found on *T. angustifolia*, but rather only on *T. latifolia* and the hybrid between these two (Eisenbach 1996, see also Chapter 1).

This study provides an interesting example of spatial variation in isotopic signature on a small scale. Looking at the $\delta^{15}N$ values for *T. latifolia*, *T. angustifolia*, and *T. x glauca* leaf tissue independent of their position in the marsh would give the impression that *Typha* species differ

considerably in isotope signature, but this difference is likely to be the result of a spatial gradient rather than differences in plant biology (see Figure 2.3). *Typha latifolia* plants were sampled from two areas about 100 m apart (see Methods, Figure 2.1). The *T. latifolia* from area B are considerably closer in $\delta^{15}N$ value to the *T. angustifolia* growing in area B than they are to the other *T. latifolia* growing in area A. The $\delta^{15}N$ values for the plants in area B are intermediate between those of the plants in area A and area C, strongly implying an environmental gradient of some kind from one end of the site to the other. Such a gradient could be caused by agricultural runoff entering the marsh at one end and diffusing across, but there is no obvious connection between the isotope values we observed and the location of creeks flowing into the marsh. It is also possible that *T. x glauca* itself alters local nitrogen dynamics in some way, as it has been shown to affect microbial communities that are relevant to nitrogen cycling (Angeloni et al. 2006). It would be interesting to know whether the differences in isotope pattern we observed are related to *Typha* physiology, or whether they are specific to this marsh and its current surroundings.

Regardless of cause, the small scale over which we observed differences in plant isotope signature underscores the importance of establishing precise baseline values. A valid baseline is essential for obtaining useful information about trophic position from $\delta^{15}N$ values (Powell 2002). However, spatial variation in isotopic signature can make establishing a valid baseline difficult, especially if it is not taken into account. As this is a common problem, there is some interest in identifying the scale over which isotopic baselines typically vary. Woodcock et al. (2012) advised that single baseline values cannot be used reliably across samples taken from >500 m apart. In our case, a distance of only about 150 m (less than the distance from area A to area C at Mud Pond; see Methods) yields a difference in $\delta^{15}N$ of approximately 3‰, which means that if

one were to use a single baseline from area A only, insects feeding on plants from area C could be interpreted as being an entire trophic level above those feeding on plants from area A.

In summary, our evidence from stable isotope analysis supports the characterization of *L. phragmitella* as an herbivore on *Typha*, and indicates that *D. julianalis* is probably relying to a large extent on protein from animal tissue. A review of the literature reveals extensive characterization of this species as a scavenger and/or predator, and we have ourselves observed it readily eating smaller *L. phragmitella* larvae in the laboratory. Although they have been touted as ecological equivalents in some work, the roles of these two Lepidopterans in cattail are actually quite different.

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CHAPTER 3

OVIPOSITION PREFERENCE AND LARVAL PERFORMANCE OF A SPECIALIST MOTH ON PARENTAL VERSUS HYBRID CATTAILS

ABSTRACT

Hybridization between distinct plant lineages produces novel genotypes that can have altered interactions with insect herbivores, resulting in changes in arthropod abundances that can impact community and ecosystem processes. Cases in which herbivores are less abundant on hybrid genotypes than on the parental species (hybrid resistance) could cause decreased biodiversity in hybrid zones, but documented cases of hybrid resistance are rare. One of the best examples of the phenomenon is the case of the specialist moth *Limnaecia phragmitella* on *Typha* spp., which has been shown to have depressed abundance on hybrid cattails (*T.* × *glauca*) relative to the parental species (*T. latifolia* and *T. angustifolia*). The purpose of this study was to investigate the mechanisms underlying this abundance pattern, and determine whether lower abundance on hybrid plants is due to female avoidance of hybrids for oviposition sites, or poor larval performance on hybrid plants. Using a combination of experiments and observational studies, we determined that female moths do not avoid ovipositing on hybrids, and in many cases prefer them over one parental, *T. angustifolia*. Parasitism rates were not high enough on hybrid plants to account for the differences in number of emerging adults. Moths emerging from hybrid plants, however, were significantly smaller than those from the parental species. Seed density was significantly lower on hybrid plants and it is likely that food limitation is responsible for the depressed abundance of this seed-feeding herbivore on hybrid cattails. We conclude that *L. phragmitella* on *Typha* does not actually provide a good example of the hybrid resistance

phenomenon. However, given the prevalence of low fertility in hybrid plant genotypes, a decreased abundance of seed-feeding herbivores may represent an important and predictable response to plant hybridization.

INTRODUCTION

Plant hybrid zones are important scenes of ecological and evolutionary action (Whitham 1999, 2006, Rieseberg et al. 2003, Arnold and Martin 2010). While interspecific hybridization is an important component of many natural systems, rates of hybridization between native and introduced species, and between wild and crop species, are a growing conservation problem (Vila et al. 2000, Ellstrand et al. 2010). One source of concern is the effect of hybridization on interactions with herbivores, and the cascading impacts these effects may have on community and ecosystem processes. Hybridization produces novel genotypes which may differ from parental species in traits that mediate insect-plant interactions, such as chemistry (Orians 2000), and plant architecture (Aguilar and Boecklen 1992, Whitham et al. 1999). These differences can affect the abundance of insect herbivores in hybrid zones (Dungey et al. 2000, Cattell and Stiling 2004, Hochwender and Fritz 2004, Bangert et al. 2006), which in turn can affect the community structure of plants and the higher trophic levels that rely on insects (e.g. Bailey et al. 2009).

Cases where herbivores are less abundant on hybrid plants ("hybrid resistance" *sensu* Fritz et al. 1994) cause decreased biodiversity and habitat quality in hybrid zones, and appear to be rare (Whitham et al. 1999, Fritz 1999). Out of 152 cases reviewed, hybrid resistance occurred in only seven (Whitham et al. 1999). Why is the hybrid resistance pattern apparently so uncommon? It is possible that hybrid resistance is underrepresented in the literature, since two-thirds of the 30 plant hybrids included in the review were confined to just a few genera (*Populus*

(9), *Eucalyptus* (6), *Quercus* (5)). It is also possible that special circumstances are required to cause hybrid resistance.

The purpose of this study is to examine a case of hybrid resistance in detail, to uncover the mechanisms underlying resistance, and to evaluate whether these circumstances are likely to occur in other hybrid systems. We focus on cattails, *Typha latifolia* (broad-leaved cattail) and *Typha angustifolia* (narrow-leaved cattail), and their hybrid, *T.* × *glauca*. The hybrid cattail *T.* × *glauca* displays resistance to the moth *Limnaecia phragmitella*, an abundant cattail specialist whose larvae develop in cattail seed heads, and which is less abundant on the hybrid cattail than on the parental species (Figure 3.1, see also Eisenbach 1996, see also Chapter 1). In this study, we investigate the relationship between *L. phragmitella* and its host plants from the insect's perspective.

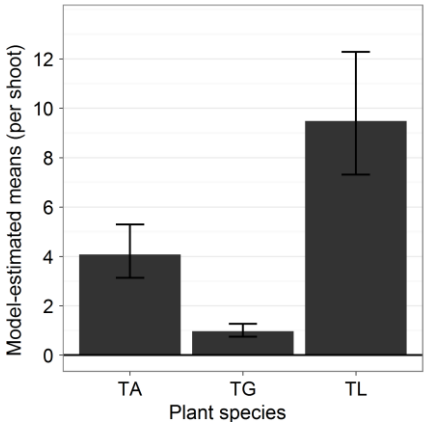

Figure 3.1. Number of *L. phragmitella* adults emerging per shoot from *T. angustifolia* (TA), *T.* × *glauca* (TG), and *T. latifolia*. Abundance on *T.* × *glauca* is depressed compared to abundance on the parental species. Data are model-adjusted means +/- 1 SE from Chapter 1.

The ability of an insect to use a particular plant depends on whether the insect recognizes the plant as a suitable host, and whether the plant is actually suitable for larval growth and development. Therefore, the relative abundance of many insects on different plants depends largely on female preference (choice of oviposition sites) or larval performance (growth,

survival, and subsequent fecundity). It seems intuitive that female insects would experience selection pressure to choose oviposition hosts that maximize offspring performance, and indeed there is strong support for this hypothesis in the literature (Gripenberg et al. 2010). However, observed correlations between female preference and offspring performance range from strong to nonexistent (Courtney and Kibota 1990, Mayhew 1997). Some cases where preference and performance do not appear correlated may result from problems in evaluating performance. For example, females may select plants that provide relatively poor nutrition (resulting in smaller larvae or slower development) but larval survival may actually be higher on such plants due to enemy-free space (Thompson 1988; Bjorkman et al. 1997). If performance is measured in the absence of enemies, females may appear to be making poor oviposition choices.

Female preference and larval performance may also be mismatched if females cannot perceive cues about plant quality (Courtney and Kibota 1990), if larvae are highly mobile (e.g. Wiklund 1984), or if females do not benefit from putting all their eggs in high-quality baskets. For example, females of the walnut fly**,** *Rhagoletis juglandis*, experience a trade-off between laying fewer eggs in high quality sites, or more eggs in lower quality sites (Nufio and Papaj 2004). If it is better to produce more offspring of somewhat lower fitness, then we should not expect a strong positive correlation between female preference and offspring performance. It is also possible that some preference and performance mismatches are maladaptive consequences of new host associations (Thompson 1988). When insects encounter novel genotypes such as introduced species and new hybrids, it is possible that preference and performance linkages are disrupted. For example, hybrids or introduced species may possess traits that females use in choosing oviposition sites, but may possess other traits making them unsuitable for larval growth and development. Examples of preference-performance mismatch have been found for native

insects on introduced plants (Ding and Blossey 2005) and on hybrid plants (Orians et al. 1997; Kokkanen et al. 2000).

The resistance of the cattail hybrid to *L. phragmitella* could thus be mediated by female avoidance of hybrids or poor larval performance on hybrids, and these need not be correlated. In addition to testing experimentally for oviposition preferences, we assessed fitness of emerging adults, larval/pupal parasitism rate, and cattail seed densities to help determine the relative roles of preference and performance in this system. This is the first study to examine a case of hybrid resistance in detail, and is a necessary step toward understanding the ecological consequences of hybridization.

METHODS

Study system

Cattails (*Typha* spp.) are widespread emergent macrophytes common in marshes, roadside ditches, and along the edges of lakes and ponds. They are ecologically dominant, highly productive, and often form dense monocultures via rhizomatous growth. Two species of cattails occur in northern North America. *Typha latifolia* (TL), the broad-leaved cattail, is native. *Typha angustifolia* (TA), the narrow-leaved cattail, was restricted to eastern coastal areas in the early 1800s, and was hypothesized to be a European introduction because it was not documented in early American floristic surveys prior to 1820 (Stuckey and Salamon 1987). The assumption of introduced status became prevalent in the literature. However, more recent evidence from pollen studies suggests that *T. angustifolia* was present in North America before European settlement, but was not widespread (Shih and Finkelstein 2008). Regardless of its origin, its range in North America has been expanding westward (Grace and Harrison 1986, Galatowitsch et al. 1999,

Smith 2000, Shih and Finkelstein 2008) and it is considered invasive in many areas. *Typha latifolia* and *T. angustifolia* hybridize to produce *T.* × *glauca*, a vigorous invasive form that spreads rapidly via vegetative growth, creates dense monocultures, and is apparently capable of out-competing both parental species especially in wetlands with artificially stabilized water levels (Smith 1987, 2000, Waters and Shay 1990, 1992, Galatowitsch et al. 1999, Zedler and Kercher 2004). The genetic status of *T.* × *glauca* has been the subject of much speculation, but a growing body of DNA work now shows that while introgression does occur at some sites (Snow et al. 2010, Travis et al. 2010, Kirk et al. 2011), the majority of hybrids appear to be F1s (Kuehn et al. 1999, Olson et al. 2009, Travis et al. 2010, Kirk et al. 2011). Thus while insects have been shown to respond differently to different classes of hybrids (F1, F2, backcrosses; Fritz 1999), *Typha* hybridization studies such as ours are largely uncomplicated by these issues, even when conducted in a field setting with naturally occurring hybrids. This study was conducted in the area surrounding Ithaca, NY (Tompkins County), where there are numerous ponds and marshes dominated by cattails, forming discrete patches that contain various combinations of parental and hybrid cattails. Although *T. angustifolia* is novel in some parts of the United States, it was common in this area at least as early as the late 1800s (Dudley 1886), so adaptive insect responses to this species and the hybrid potentially exist.

Typha plants are associated with a diverse insect assemblage, including the moth *Limnaecia phragmitella*[5] (Cosmopterigidae) (Claassen 1921, also see Chapter 1). *Limnaecia phragmitella* (Figure 3.2) is a cattail specialist with a worldwide distribution (Stainton 1870, Meyrick 1888, 1895, Busck 1901, Covell 1984), though it has been suggested that it may have

[5] Taxonomy note: This species was originally described as *Limnaecia phragmitella* by Stainton (1851). He later moved it to the genus *Laverna* (Stainton 1858). It was subsequently moved back to *Limnaecia* by Meyrick (1888). Dyar et al. (1902) misspelled the genus as *Lymnaecia* in his checklist. The mistake was perpetuated by Claassen (1921), Eisenbach (1996), and others.

been introduced to Australia (Common 1990) and it was not documented in North America until 1901 (Busck 1901). Adults have a 15-20 mm wingspan and fly from June-August. On warm nights they can be observed in great numbers in cattail marshes, running along leaves and stalks and flying low above the plants. Females oviposit directly into the developing flowerheads, wedging the tip of the abdomen into the tightly-packed flowers and laying a single egg in several different places and on different plants. Eggs hatch in about a week and the larvae begin feeding within the flowerhead, first feeding on flowers and later, on seeds (Classen 1921). Isotope analysis has confirmed that the moth is indeed an herbivore (see Chapter 2).

It has been hypothesized that the larvae secrete silk that binds the cattail fluff so that in the autumn, when the fluff would normally dehisce and carry seeds on the wind, it remains attached to the stalk as a puffy mass (Claassen 1921). The *L. phragmitella* caterpillars overwinter inside the puffy seed heads, and pupate either there or in a burrow in the stem (von Heyden 1863, Claassen 1921). During the fall and winter various instars, including full-grown larvae, can be found in the same seedhead (personal observation). By late May in Ithaca, NY, all larvae have pupated, and adults begin emerging in mid-June. Dispersal behavior of the moth has not been studied.

Figure 3.2. *Limnaecia phragmitella* egg, larva, pupa, and adult.

I. Assessment of Moth Fitness

Fitness of *L. phragmitella* reared from *T. latifolia*, *T. angustifolia*, and *T.* × *glauca* was assessed via weights of adult moths collected upon emergence. These moths were collected as part of a larger survey of cattail insects (see Chapter 1), although the weights have not been previously reported. In May 2006, senesced cattail shoots from the previous year were collected haphazardly from 8 sites in and around Ithaca, NY. At each site, we identified sampling areas where only one cattail species was growing, as determined by field characters (Smith 2000). In general, the cattails were well segregated and we simply avoided areas of overlap where two species grew near each other. Sampling areas covered approximately 200 m^2. In small sites, the sampling area encompassed all the area occupied by a given species. The plants at these sites (though not the same shoots collected for this study) were genotyped using microsatellite DNA analysis as part of the larger survey (see Chapter 1) to verify parental/hybrid status. In that study,

all putative *T. angustifolia* and *T. latifolia* were correctly identified and all putative *T.* × *glauca* were F1 hybrids.

We collected 30 shoots of each species at each site, or as many shoots as possible if there were fewer than 30 shoots remaining with the seed head still attached to the stem. Seed heads were put in Ziploc bags, and stems were bagged in plastic tubing. The tubing was rolled at the top and bottom and secured using staples at the bottom and a binder clip at the top (so that the bag could be opened and closed again easily). Heads and stems were hung in the laboratory, with natural daylight. There was no air conditioning, but two fans helped cool the room on particularly hot days. The bags were checked every few days, and all living adult *L. phragmitella* were collected and frozen, and later weighed. Individuals that were found dead in the bags were not weighed. Moths emerged between 7 June and 28 July 2006.

Data were analyzed using a mixed effects model with site and individual shoot as random effects and cattail species, moth sex, and their interaction as fixed effects. Analyses were performed in R using the lmer() function in the lme4 package (Bates et al. 2011). Contrasts were performed using the estimable() function in the gmodels package (Warnes 2011).

II. Oviposition Preference Experiments

Oviposition preference tests were conducted in large outdoor insect cages, using moths reared from senesced cattail shoots of known species, and freshly-cut cattail shoots from the field. In 2005, we conducted a choice experiment in which moths were presented with *T. angustifolia*, *T.* × *glauca*, and *T. latifolia* shoots. In 2007, we conducted a no-choice experiment in which moths were presented with a single cattail shoot. In both experiments, preference was assessed by observing oviposition behavior.

Rearing and handling moths

Moths for both oviposition experiments were obtained by collecting senesced cattail shoots in early June (when *L. phragmitella* has begun to pupate) and allowing the moths to emerge naturally from the shoots inside large insect cages. The senesced shoots were collected from a variety of sites in and around Ithaca, NY, and separated by plant species (TL, TA, and TG) into three large insect cages (4x4x2m; the "stock cages") assembled on vinyl sheets rather than on a natural substrate such as grass, which would have made it difficult to find the adult moths for the experiment. We found in previous work that keeping the shoots upright and well-spaced was important for maximizing successful moth emergence. Therefore, the senesced shoots (300-700 of each species) were supported upright by inserting the stems into the holes of a long piece of poultry wire suspended horizontally across one side of the cage. Stems were collected as well as seed heads because a substantial fraction of *L. phragmitella* larvae pupate in the stems (personal observation).

To provide natural stimuli for courtship and mating prior to the oviposition tests, 5-10 flowering cattail plants were dug and placed in 11L pots in the stock cages. Until the test, moths were exposed only to plants of the cattail species from which they were reared.

On the day of each trial, the requisite number of female moths was caught using clear plastic condiment cups (one moth per cup). It is easy to distinguish male and female moths on sight, especially once captured. The moths were dyed with different colors of fluorescent powder (which glows brightly under UV light) to make it possible to use more than one moth per cage in the experiment and still distinguish individuals (see Appendix, Figure 3.A1). The fluorescent powder did not appear to affect the moths' behavior. The dyed moths were released

into the test cages by early afternoon, giving them several hours to acclimatize before testing began that night.

A. Choice Experiment

Experimental setup

Three large insect cages (4m x 4m x 2m) were used for the trials. Each cage contained 6 female moths reared from the same host plant species, and dyed six different colors with fluorescent powder (red, yellow, blue, orange, pink, and green). New moths were used each night. Shortly before each trial, three cattail shoots of each species were cut at ground level from the field, and placed in jars of water in the cages. The shoots were fastened to stakes to keep them upright. Each cage had one shoot of each cattail species (see Figure 3.3a). The positions of the cattail shoots within the cages were rotated each night. On a given night, the cattails all came from the same site, but over the course of the experiment we used cattails from several different sites.

Testing procedure

All trials were started at civil twilight. The experimenter quietly entered the cage and shined a UV flashlight onto the three test plants to look for moths. Moths were recorded as being on the leaves, on the flower head, or in the process of ovipositing into the flower head. Oviposition for this species is a clearly visible process. Females remain on the flower head for some time before and after ovipositing, and usually oviposit several times in different places on the same head. Moths did not usually appear disturbed by the observations. Data were taken every half hour for a total of 5 time points each night. After the trial, the moths were removed from the cages and not reused. The experiment was conducted for 10 nights.

Data analysis

Data were analyzed by multinomial logistic regression in R (R Development Core Team 2012) using the mlogit() function of the mlogit package (Croissant 2011), with the trials for moths from each host origin modeled separately. In multinomial logistic regression, the choice of an individual moth is modeled as a combination of binary logistic regressions, with a separate equation for each potential choice outcome (TA, TL, or TG).

B. No-choice experiment

Experimental setup

Nine smaller insect cages (2m x 2m x 2m) were arranged in 3 blocks of 3 (Figure 3.3b). Each cage housed three moths reared from the same cattail species and dyed three different colors (red, blue, yellow) using fluorescent powder. Shortly before each trial, three shoots each of *T. latifolia*, *T. angustifolia*, and *T.* × *glauca* were cut at ground level and one shoot was put in each cage. The shoots were kept in water and supported upright by small stands made for the purpose. The cattails were collected from a variety of different natural sites. All three cages in one block received the same species of plant, but the plant species assignments were rotated among the blocks each night. Leaf samples were taken from all plants for genotyping.

Testing procedure

All trials were started at civil twilight. From the outside of each test cage, the experimenter shined a UV flashlight to determine whether there were any moths on the cattail shoot and whether they were actively ovipositing. We repeated this procedure at half-hour intervals for a total of 6 observations. At the end of the trial, we inspected the head of each shoot with the UV flashlight and looked for fluorescent powder marks on the head, since the moths

usually left evidence if they landed on the head at all during the trial (see Appendix, Figure 3.A1). Sometimes moths left marks characteristic of ovipositing behavior (trails of distinct dots indicating a female inserting her abdomen repeatedly before selecting a final location). After the trial, the moths were removed from the cages and not reused. Moths that were never seen in the cage at all during the trial, or were found dead at the end of the trial were eliminated from the analysis (occasionally a moth drowned in a puddle, was caught by a spider, or was accidentally killed in some other way). The experiment was conducted for 17 nights.

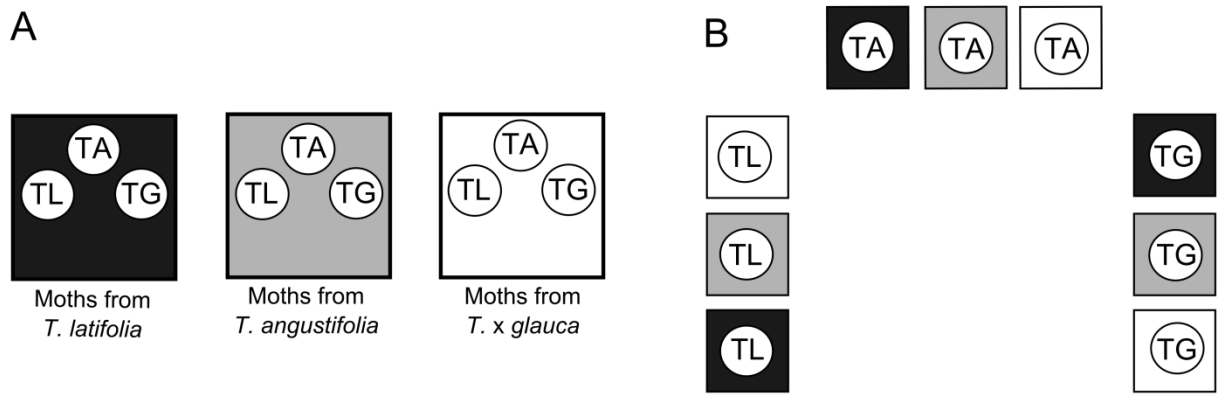

Figure 3.3. Experimental setup diagrams for the choice (A) and no-choice (B) experiments. Each box represents an insect cage with dimensions given in the text. The species names of the test plants are abbreviated inside circles. Cattail species were rotated each night within the cages (choice exp.) or among the blocks of three cages (no-choice exp.). The boxes are shaded according to the species of cattail from which the moths in that cage were reared, i.e. moth origin (black= *T. latifolia*, gray= *T. angustifolia*, and white = *T* x *glauca*). Moth origin was not rotated among cages.

Data analysis

Data were analyzed by logistic regression in R using the glm() function with a binomial distribution and a logit link (R Development Core Team 2012). Contrasts were performed using the estimable() function in the gmodels package (Warnes 2011). Host acceptance (0,1) was modeled as a function of plant species in the test cage, host plant of moth origin, temperature as a continuous variable, and the interaction of test plant and host plant. Ambient temperature was measured at the start of the trial.

Genotyping

Tissue samples from all test plants in the no-choice experiment were genotyped using microsatellite markers to verify parental/F1 hybrid status. Tissue was lyophilized and then ground using a GenoGrinder. Ground tissue was returned to –20 C until extraction. DNA was extracted with plant DNeasy kits (Qiagen, Inc. City State), using the basic miniprep protocol. We used 7 primers developed for one of the parental cattail species, *T. angustifolia* (TA3, TA5, TA7, TA8, TA16, TA20) (Tsyuko-Omeltchenko et al. 2003) and tested in North American cattails (Snow et al. 2010). These primers amplify in *T. latifolia* as well, and appeared likely to amplify distinct alleles in the two parental species in our study populations. F1 individuals would be characterized by having one allele from each parental species at each locus. Advanced hybrids and backcrosses would display a combination of species-specific loci and mixed-species loci. The probabilities of misidentifying F2 or first-generation backcrosses using 6 loci, assuming Mendelian inheritance, are given in Table 3.1. Based on these probabilities, we determined that using 6 alleles was sufficient.

Table 3.1. Probabilities associated with misidentification of backcross or F2 individuals based on 6 microsatellite loci.

Actual Genotype	Apparent Genotype	Probability
Backcross	Pure parental	0.016
Backcross	F1	0.016
F2	Pure parental	0.0005
F2	F1	0.016

The 5' end of each primer was fluorescently labeled with NED (TA3, TA20), VIC (TA5, TA16), 6FAM (TA7) or PET (TA8). PCR was performed using Multiplex PCR kits (Qiagen, Inc. City State), using the standard protocol with Q solution, except that the ratios of the primers in the primer mix were optimized (volume per reaction was increased from 0.2 to 0.3 µl for TA5, and decreased to 0.15 for TA16 and TA20). PCR was performed using the following cycling

parameters: 95° for 15 min; 7 x (94° C for 30s, 57° C for 1 min 30s (-1° C per cycle)); 72° C for 1 min; 25 x (94° C for 30s, 50° C for 1 min 30s, 72° C for 1 min; 72° C for 10 min. Genotyping was performed using an ABI capillary sequencer in the Evolutionary Genetics Core Facility at Cornell University. Data were collected and scored manually using Genemapper v. 3.0 (Applied Biosystems).

III. Seed Density Comparisons

To determine whether differences in numbers of seeds among *T. latifolia*, *T. angustifolia*, and *T.* × *glauca* could account for differences in the abundance of *L. phragmitella*, we sampled cattail pistillate spikes (flower heads) from 7 sites and estimated the number of seeds per flower head, as well as the density of seeds for each plant. Total numbers of plants were 31 *T. latifolia*, 20 *T. angustifolia*, and 21 *T.* × *glauca.* We chose sites that were included in the arthropod abundance survey (see Chapter 1) to facilitate our comparisons.

Flower heads were collected in August (most in 2006, some in 2009), when the seeds were formed but not fully mature, and the fluff was not yet dehiscent. The seeds at this stage of development are bright yellow and clearly visible amongst the white fibers of the fluff. We measured the dimensions of each head and then took a 0.5 cm cross section from the center of each head (for *T. latifolia* plants, we used only one half of this section). The fluff from each section was teased apart in a 12x16cm tray using forceps and a piece of stranded copper wire untwisted at the end to form a tiny wire rake. The tray was lined with white paper and divided into four quadrants. Once the fluff was spread sufficiently, we took a digital photo. We used Paintshop Pro 7 (Jasc Software) to increase the brightness of the image by setting the color of the paper in the background to pure white. Then we modified the color curves to increase the

saturation of the yellow seeds compared to everything else in the photo, making them stand out distinctively from all other flower parts even when partly obscured. The seeds in the photo were then counted manually.

We used the number of seeds in each section and the length of the flower head to calculate an estimated number of seeds per head. We used the length and circumference of the central axis to calculate the surface area in each head available for floral attachment, and used this to calculate seed density (seeds per cm^2).

Seed densities were analyzed using a mixed effects model with site as a random effect and cattail species as a fixed effect. Analyses were performed in R using the lmer() function in the lme4 package (Bates et al. 2011). Contrasts were performed using the estimable() function in the gmodels package (Warnes 2011).

To determine whether seed density could be responsible for the *L. phragmitella* abundance pattern, data on *L. phragmitella* abundance and body mass was obtained from the arthropod survey reported in Chapter 1, as described above in the methods for the Larval Performance Assessment. The data consisted of numbers and masses of LP adults reared from senesced plants from the same sites sampled for the seed counts. For this analysis, we summed the body masses of moths from each shoot to obtain the biomass produced per shoot. The dimensions of the central axis of each flowerhead had been measured as part of the original survey, but these data were not presented in the report of the survey results. Here, we used the dimensions of the central axis to convert biomass per shoot to biomass per cm^2 axis surface area, for comparison with the seed densities.

The *L. phragmitella* biomass (mg/cm^2) data were analyzed using a mixed effects model with site as a random effect and cattail species as a fixed effect. Analyses were performed in R

using the lmer() function with a Poisson distribution in the lme4 package (Bates et al. 2011). Since the Poisson requires integer values, we transformed the data by multiplying by 10,000 and rounding to the nearest whole number. Contrasts were performed using the glht() function in the multcomp package (Hothorn et al. 2008).

IV. Parasitism Rates

Eisenbach (1996) reported higher parasitism rates for *L. phragmitella* on *T.* × *glauca* than on the parental species. To determine whether differences in parasitism rates could be driving the hybrid resistance pattern, data on parasitoid abundance were obtained from the arthropod survey reported in Chapter 1, as described in the methods for the Assessment of Moth Fitness (above). In that study, parasitoids were collected as they emerged in the laboratory from field-collected cattail shoots. Thus, the parasitoids were not reared directly from *L. phragmitella* larvae. For this analysis, we used the data for all parasitoid species found in the survey with more than 10 individuals and known to target Lepidoptera: *Hymenochaonia delicatus* (Braconidae), *Temelucha gracilipes* (Ichneumonidae), *Scambus* sp., probably *S. hispae* or *S. decorus* (Ichneumonidae), *Chelonus* sp. (Braconidae), *Apanteles* sp. (Braconidae), and several Chalcidoid wasp species that were commonly found inside *L. phragmitella* cocoons and stem burrows. We also included *Eupelmus dryorhizoxeni*, which is a hyperparasitoid that has been recorded from *Apanteles* (Gibson 2011), therefore it is likely that the *E. dryorhizoxeni* collected in our study came indirectly from *L. phragmitella.* These parasitoids represent the vast majority of parasitoids collected in the study.

These parasitoids probably target a range of Lepidoptera species, but in this system the only possible hosts were *L. phragmitella* and a Crambid moth of similar size and habits,

Dicymolomia julianalis. *Dicymolomia julianalis* is not found on *T. angustifolia*. Therefore, we were able to assume that all Lepidoptera parasitoids emerging from *T. angustifolia* plants were from *L. phragmitella* hosts. Furthermore, while some *L. phragmitella* larvae pupate in the stem, *D. julianalis* always remains in the seed head. Thus, all Lepidoptera parasitoids emerging from the stems must have come from *L. phragmitella* (for the survey, heads and stems were bagged separately in the laboratory, though head/stem data were not presented in the report of the survey results). Only parasitoids emerging from *T.* × *glauca* seed heads and *T. latifolia* seed heads could possibly have come from *D. julianalis.* Using this reasoning, we were able to calculate the *maximum possible effect* parasitoids could have had on the *L. phragmitella* abundance pattern by assuming that all parasitoids emerging from *T.* × *glauca* seed-heads came from *L. phragmitella*, and all parasitoids emerging from *T. latifolia* seed-heads came from *D. julianalis.* We adjusted the raw *L. phragmitella* data to account for parasitoid mortality, considering each parasitoid to have replaced one *L. phragmitella* individual, except for the Chalcidoids, which emerge from a single host in groups of approximately six (pers. obs.). Then we re-ran the species abundance analysis from the survey (Chapter 1) on the parasitoid-adjusted data to obtain model-estimated means for adjusted *L. phragmitella* abundance.

RESULTS

I. Assessment of Moth Fitness

For females, the moths that developed on hybrid cattails were 25% smaller than moths from *T. latifolia* ($p<.001$) and 11% smaller than moths from *T. angustifolia* ($p=.024$). Moths from *T. latifolia* were significantly larger than moths from *T. angustifolia* ($p<.001$). (See Figure 3.4A).

For males, the moths from *T. latifolia* and *T. angustifolia* were not different in size (p=.722), but moths from hybrid cattails were 32% smaller than moths from *T. angustifolia* (p<.001), and 31% smaller than moths from *T. latifolia* (p<.001). (See Figure 3.4B).

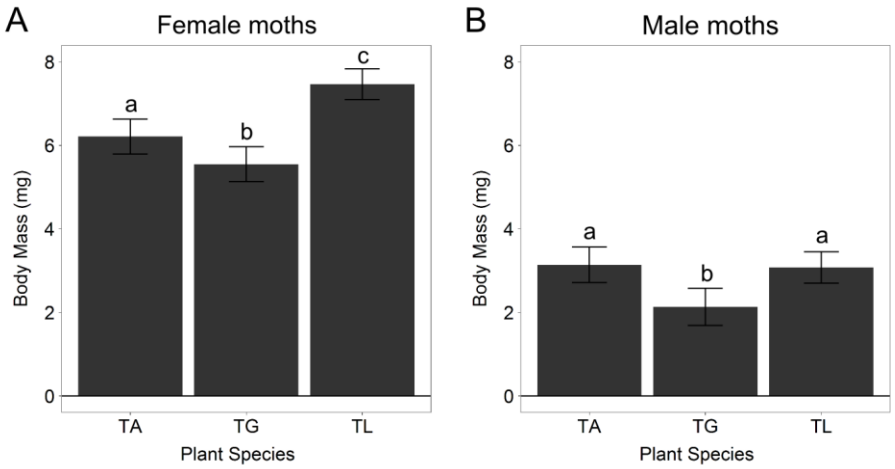

Figure 3.4. Body mass (mg) of *L. phragmitella* reared from *T. latifolia*, *T. angustifolia*, and *T.* × *glauca*. Data are model-estimated means ± 1 standard error.

II. Oviposition Preference Experiments

A. Choice Experiment

Of 175 marked moths, 64 were seen on the head of a test plant during the trial and were therefore considered to have chosen that plant as an oviposition site. Moths that did not choose a plant were eliminated from the study. Of the moths that made a choice, most were observed on only one plant during the trial. Relatively few (12.8%) were observed on two different plants. Data from all moths are included in Figure 3.5, which is a summary of the raw data showing the percent of moths from each host origin that chose each cattail species or combination of species. However, because of the limitations of multinomial logistic regression, moths that chose more than one species were not included in further statistical analysis.

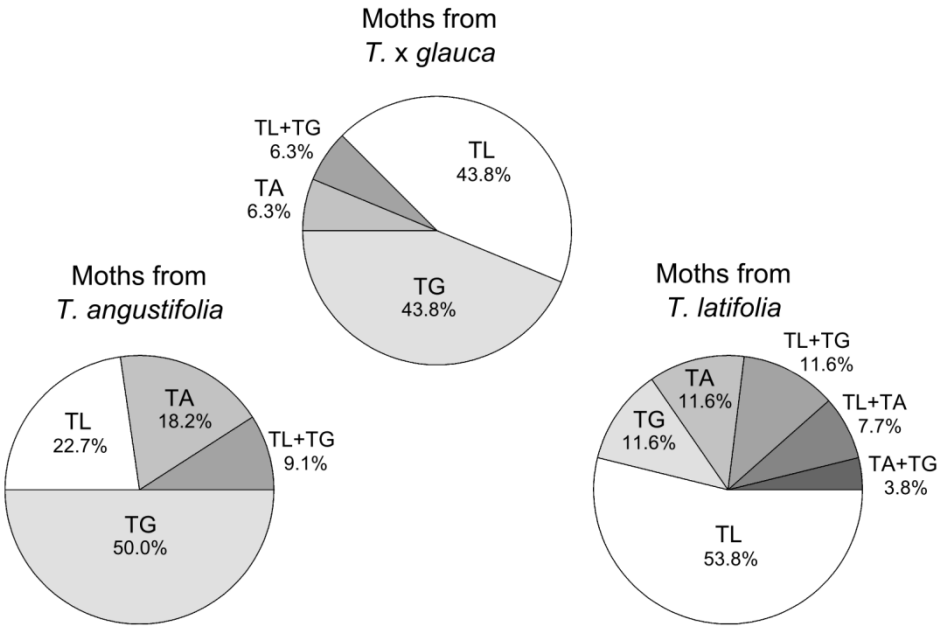

Figure 3.5. Piecharts showing the actual oviposition choices of moths from each host origin. Moths that chose more than one plant were not included in further statistical analysis using multinomial logistic regression.

Multinomial logistic regression showed that most contrasts were nonsignificant, though some trends were suggestive. The probabilities associated with each plant choice for moths originating from TL, TA, and TG are shown in Figure 3.6. TA moths tended to prefer TG over TA (p=0.083), but not over TL (p=0.14). TG moths seemed to prefer TG and TL over TA (p=0.069). TL moths significantly preferred TL over TG and TA (p=0.015). At least one moth from every origin chose each species, indicating that host preferences are not absolute.

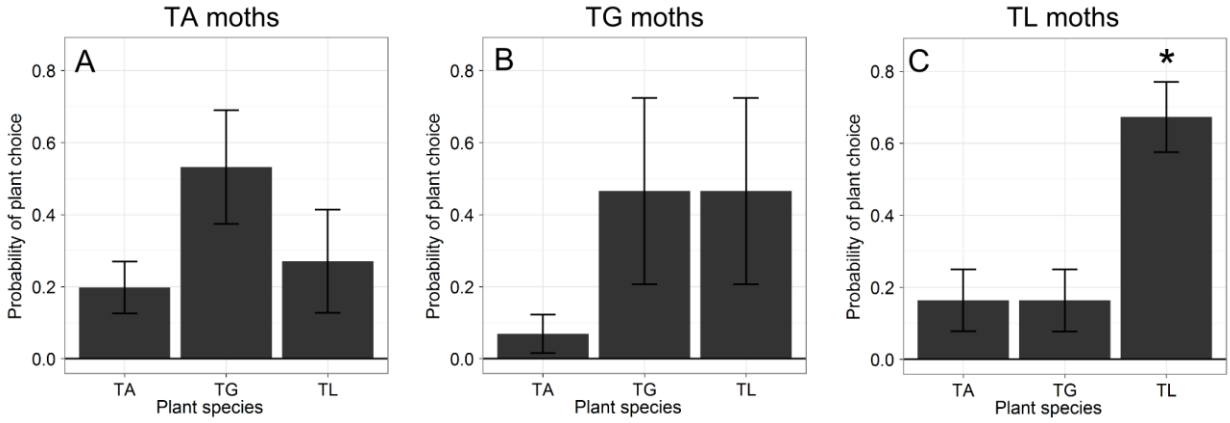

Figure 3.6. Model-estimated probabilities associated with each plant choice for moths originating from TL, TA, and TG. Data are means ± 1 SE. The asterisk (*) indicates the significant contrast.

B. No-choice Experiment

Genotyping revealed no evidence of advanced hybridization in the test plants (backcrosses, F2 hybrids, etc), and all test plants were correctly identified as parental or F1 hybrids. See Appendix for genotyping results (Table 3.A1).

Host acceptance was significantly affected by cattail species, moth origin, and was strongly influenced by temperature ($p<0.001$). Figure 3.7 displays model estimated probabilities of host acceptance by moths originating from *T. latifolia*, *T. angustifolia*, and *T.* × *glauca* as a function of temperature. We found no evidence for host preference in moths reared from TA or TG (all contrasts involving TA and TG moths were nonsignificant with $p> 0.222$). Moths from TL were significantly more likely to accept TG as a host than TA ($p=0.005$); the odds of accepting TG were 3.6 times higher than the odds of accepting TA.

Numerically, the odds of a TL moth accepting the natal host (TL) were intermediate between the odds of accepting TG and TA, but those differences were not significant ($p=0.188$ for TG; $p=0.155$ for TA).

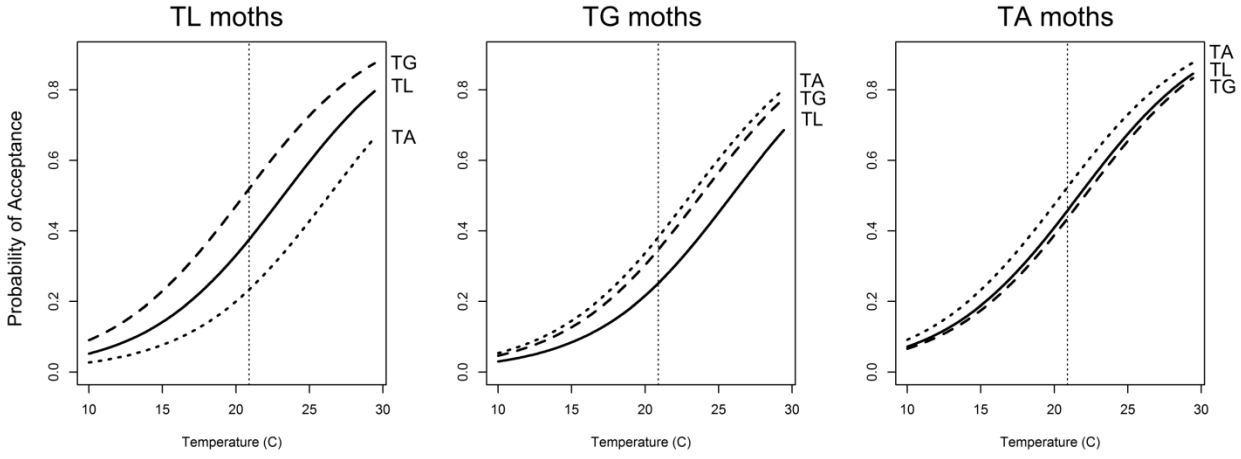

Figure 3.7. Model estimated probabilities of host acceptance by moths originating from *T. latifolia*, *T. angustifolia*, and *T.* × *glauca* as a function of temperature. Each graph contains three curves, showing the probability of acceptance for each plant species. The dashed line is TG, the dotted line is TA, and the solid line is TL. Mean temperature across all trials is plotted as a vertical dotted line.

III. Seed Density Comparisons

We found that *T. latifolia* has more seeds per head ($p<0.001$), and higher densities of seeds ($p<0.001$), than either *T. angustifolia* or *T.* × *glauca*. The numbers of seeds in *T. angustifolia* and *T.* × *glauca* were only marginally different ($p=0.096$), but after accounting for the smaller size of *T. angustifolia* heads, the densities (seeds/cm^2) are significantly different ($p<0.001$). See Table 3.2 and Figure 3.8A.

Table 3.2. Estimated total number of seeds per head for *T. latifolia*, *T. angustifolia*, and *T.* × *glauca*.

Species	Mean Seeds	SE	N
T. latifolia	63480	5987	31
T. angustifolia	17402	6854	20
T. × *glauca*	2589	6491	21

Densities of *L. phragmitella* followed the same pattern as seed densities (Figure 3.8B). *T. latifolia* had the highest density of moths, followed by *T. angustifolia*. *T.* × *glauca* had the lowest density. All contrasts were highly significant ($p<0.001$).

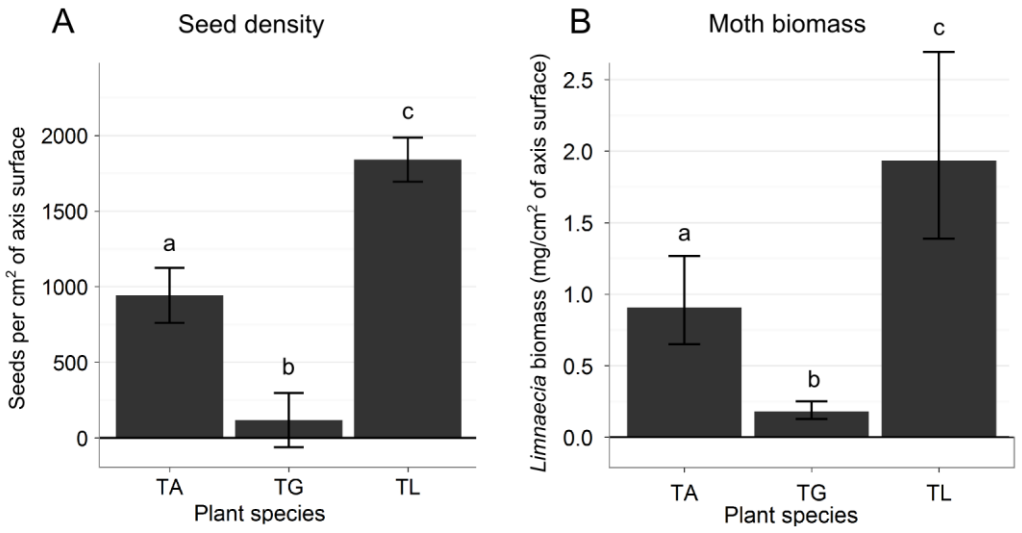

Figure 3.8. (A) Estimated densities of seeds (seeds/cm^2 of axis surface area) for *T. angustifolia* (n=20), *T.* × *glauca* (n=21), and *T. latifolia* (n=31) seedheads combined from all 7 sites. Data are means ± 1 SE. (B) Densities of *L. phragmitella* adults reared (number of moths produced per cm^2 of axis surface area) from *T. angustifolia, T.* × *glauca*, and *T. latifolia* shoots from the same 7 sites used in the seed count study. Data are means ± 1 SE.

IV. Parasitism Rates

The overall parasitism rate of *L. phragmitella* on *T. angustifolia* was 9.4% (63 parasitized/670 total). The parasitism rate on *T. latifolia* was between the maximum possible rate of 16.7% (597/3571), assuming all parasitoids from the seed head came from *L. phragmitella*, and the minimum possible rate of 8.7% (284/3258), assuming all parasitoids from the seed head came from the alternative possible host *D. julianalis*. The maximum possible rate on *T.* × *glauca* (16.2%, 56/345) was higher than the rate on *T. angustifolia*, but similar to the maximum rate on *T. latifolia*. The minimum rate on *T.* × *glauca* (5.2%, 16/305) makes it potentially the lowest. However, since the alternative possible host, *D. julianalis*, is much less abundant than *L. phragmitella*, the actual parasitism rates on *T. latifolia* and *T.* × *glauca* are probably closer to the maximum possible rates than to the minimum ones.

Regardless, these differences parasitism rates do not translate to large changes in the number of *L. phragmitella* even when the maximum possible effect of parasitism is assumed (Figure 3.9). Assuming the highest possible rate on *T.* × *glauca*, and the lowest possible rate on *T. latifolia* (the rate on *T. angustifolia* is known), the *L. phragmitella* hybrid resistance pattern remains unchanged.

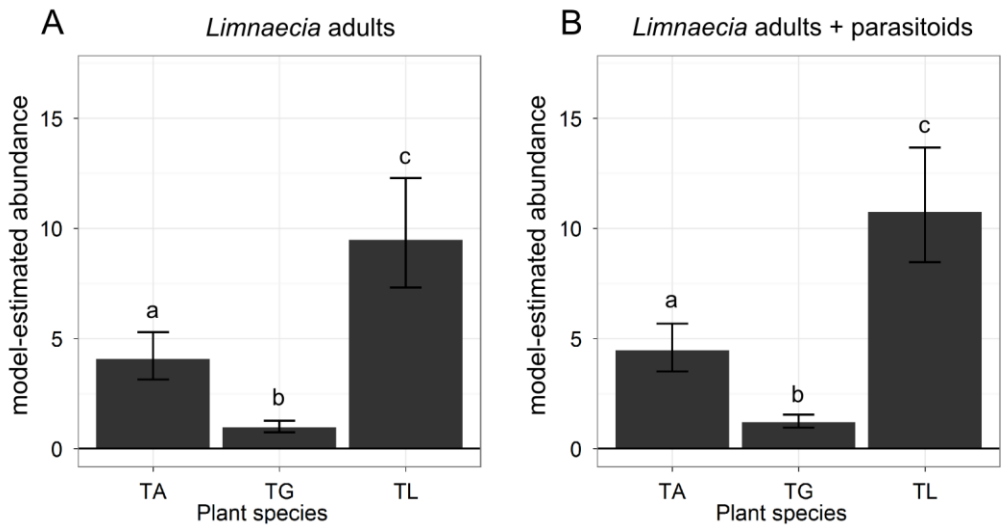

Figure 3.9. Model-estimated means ± SE for number of *L. phragmitella* on *T. angustifolia*, *T.* × *glauca*, and *T. latifolia* per shoot. Panel A shows the abundance pattern based on the number of *L. phragmitella* adults that successfully emerged (from Chapter 1). Panel B shows the abundance pattern that results from the model when numbers of parasitoids are added to the *L. phragmitella* totals, assuming the minimum possible parasitism rate on *T. latifolia*, and the maximum possible rate on *T.* × *glauca*.

DISCUSSION

Limnaecia phragmitella on *Typha* spp. is known as a classic case of hybrid resistance (Eisenbach 1996, Fritz 1999, Fritz et al. 1999, Whitham et al. 1999). Not only are the moths less abundant on their hybrid host plant, but this study showed they are also smaller. In female moths, body mass is directly related to fitness (Honek 1993). For many insect taxa, including Lepidoptera, a 1% increase in female body mass corresponds to approximately 1% increase in fecundity (Honek 1993). For example, in the Tortricid moth *Cnephasia jacatana*, a 25% decrease in female body mass was estimated to result in a 25-27% decrease in the number of fertile eggs (Jimenez-Perez and Wang 2004). In our study, the differences in body mass (Figure 3.4) are not as pronounced as the differences in abundance (Figure 3.1), but this is likely due to physiological constraints on body size.

Lower body mass corresponding to lower density of individuals is not necessarily an expected result since low densities could in some systems result in release from intraspecific

competition and hence large body mass (e.g. Podoler 1974, Lamberti et al. 1987, Gibbs et al. 2004). In the gypsy moth, increased larval density results in smaller larvae even when food is not limited, presumably due to stress (Reilly and Hajek 2008). Some insects engage in facultative cannibalism that can result in larger body size of the cannibals (e.g. Joyner and Gould 1985), as well as a reduction of larval density. None of these situations appears to be the case for *L. phragmitella*, since high densities corresponded to larger larvae in our study.

Comparing Figure 3.8A and 3.8B, it is clear that *L. phragmitella* biomass shows the same pattern as seed density, strongly implying that food limitation is the primary factor causing lower *L. phragmitella* abundance and body mass on hybrid cattails, and possibly explaining the difference in average density and body mass between *T. latifolia* and *T. angustifolia* as well. In an earlier study, Eisenbach (1996) estimated the number of seeds produced by the same two cattail species and their hybrid at two sites in Michigan. Although he did not present seed count data explicitly, he did present mean values for flower density and percent of flowers with seeds. He found that *T. latifolia* had significantly higher values for these measures than *T. angustifolia* and *T.* × *glauca*, which were not significantly different. In our study, seed densities on all three cattails were different, and on average the hybrid had considerably lower seed abundance and density than either of the two parental species. Eisenbach concluded that seed availability did not appear to explain the difference in *L. phragmitella* abundance between *T. angustifolia* and *T.* × *glauca* in his study, and speculated that *L. phragmitella* might have evolved oviposition preferences for parental cattails because the seed supply on hybrid cattails is notoriously variable (Smith 1967).

Our study shows that oviposition preference actually plays little role in determining *L. phragmitella* abundance, and certainly could not explain higher abundance on *T. angustifolia*

than the hybrid. Moths from *T. latifolia* seemed to prefer *T. latifolia* when they were given a choice, though in the no-choice experiment, the hybrid was accepted as a host at least as often as *T. latifolia*. In a landscape of mixed cattail species, most moths will have come from *T. latifolia* because the moth is so much more abundant on *T. latifolia*. Moths from hybrid plants are relatively few, and they show no preference for the hybrid over *T. latifolia.* So female preference may help explain why there are more moths on *T. latifolia* than the hybrid or *T. angustifolia*. It cannot explain the difference between *T. angustifolia* and the hybrid, since no moths showed any preference for *T. angustifolia* over the hybrid. Even moths reared from *T. angustifolia* seemed to prefer the hybrid, and *T. latifolia* moths either didn't differentiate between *T. angustifolia* and the hybrid, or else they preferred the hybrid over *T. angustifolia*, which is counter to the hybrid resistance pattern.

Eisenbach (1996) further hypothesized that higher parasitism rates of *L. phragmitella* on hybrid cattails could explain their low abundance there, and he found some evidence of higher parasitism rate on *T.* × *glauca*. Our study was not designed to determine an exact parasitism rate, but our results do not contradict Eisenbach's (within the range of possible parasitism rates we determined, it is possible that the parasitism rate on *T.* × *glauca* is somewhat higher than on the parental species). However, the numbers of *L. phragmitella* larvae on *T.* × *glauca* are so small compared to the numbers on *T. latifolia* and *T. angustifolia* that the differences in parasitism rate do not translate to substantial differences in the number of parasitized larvae on each host. Thus, based on the parasitism rates in our study, larval mortality due to parasitism cannot account for the lower abundance of *L. phragmitella* on *T.* × *glauca.* In fact, a rough calculation based on the data summarized in Eisenbach (1996) shows that those parasitism rates are similarly insufficient to alter the *L. phragmitella* abundance pattern presented in that paper (Figure 3.10).

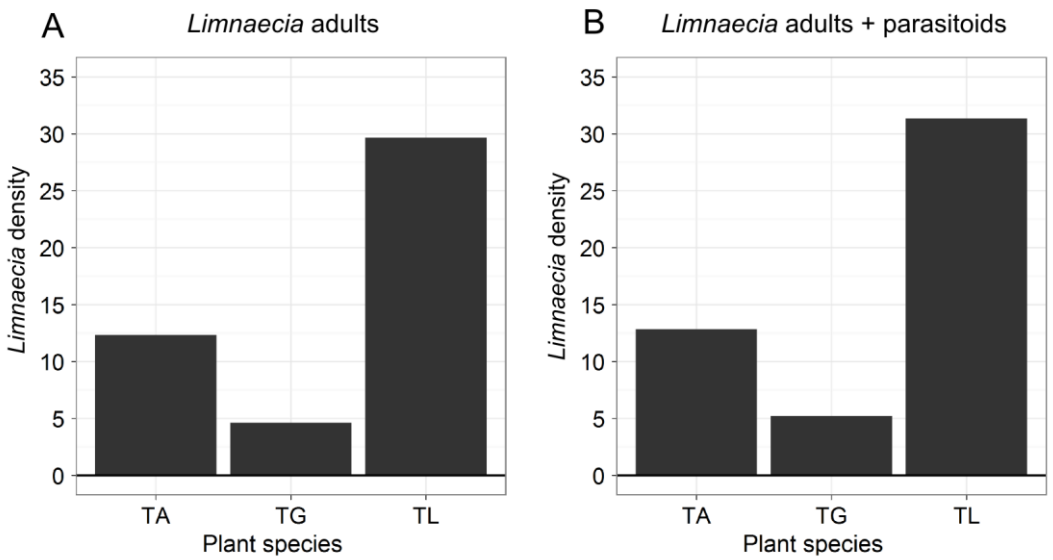

Figure 3.10. (A) Numbers of *L. phragmitella* adults emerging per average-sized cattail head from Eisenbach (1996), averaged across the three sites and two years encompassed by that study (the general pattern was the same at all sites and in both years). Data are means obtained from Figure 1A and 1B in Eisenbach (1996). (B) The data from panel A, adjusted by us to include parasitized larvae based on the parasitism rates presented in Table 4 in Eisenbach (1996), averaged for both years of the study.

Interspecific hybrids often display reduced fertility (Mayr 1992, Ellstrand and Elam 1993, Coyne and Orr 1998, Burke and Arnold 2001). In plants, hybrid incompatibilities are often manifested by reduced seed set (e.g. Heiser 1947, Grant 1966, Ramsey et al. 2003, Song et al. 2004, Tauleigne-Gomes and Lefebvre 2008, Marques 2011, Zhang et al. 2011). Thus, we might expect seed-feeding herbivores to display depressed abundance on hybrid host plants that display low fertility. To our knowledge, no studies have compared abundance patterns of seed feeders vs. other herbivores on hybrid plants, though such a comparison would be very interesting. However, cases of depressed abundance of seed feeders on plants with low fertility are not really examples of "hybrid resistance" in the original sense of the term (Fritz 1994, Fritz 1999 et al., Whitham et al. 1999). A failure to produce sufficient seed to support large abundances of seed-feeding herbivores is clearly not a resistance trait that could confer greater relative fitness to individuals with the trait. In addition, lower herbivore abundance on the hybrid relative to the parentals does not necessarily imply higher relative fitness. On a hybrid with a high rate of seed

sterility, relatively few seed-feeders could potentially eat all the seeds produced, decimating the fitness of the plant. Although the herbivore may be less abundant on the hybrid than on the parental, its effect on plant fitness could be equally detrimental.

Fritz et al. (1994, 1999), Whitham et al. (1999) and others (e.g. O'Reilly-Wapstra et al. 2005) have advocated using hybrid plant systems as tools for understanding the genetic basis of plant resistance mechanisms. This involves the identification of cases in which hybrid plants display resistance traits such as novel combinations of defensive compounds, or genetic disruption of plant traits herbivores recognize. For such studies, *L. phragmitella* on *Typha* spp. does not appear to be a useful example of an herbivore-resistant hybrid. Thus, what Fritz (1999) called, "the strongest evidence for hybrid resistance," should not be used to make generalities about altered resistance of hybrid plants. Fritz (1999) suggested that hybrid resistance might occur when parental species display dominant resistance traits that act synergistically in F1 progeny, and proposed that the comparative rarity of hybrid resistance would support the hypothesis that resistance traits tend to be recessive. If *L. phragmitella* is not a relevant example of hybrid resistance, then the phenomenon may be even less common than previously believed.

This is not to say that the *L. phragmitella* system is unimportant as an example of an insect response to hybrid host plants. In fact, depressed abundance of seed-feeding insects on hybrids probably represents an important, and perhaps predictable, consequence of hybridization. Two other insects on *Typha*, the Lygaeid bug *Chilacis typhae* and the Crambid moth *Dicymolomia julianalis* have been described in the literature as seed feeders (Claassen 1921), but displayed an intermediate abundance pattern in our previous work (see Chapter 1). The Lygaeid occurred in much lower abundance on the hybrid than on *T. latifolia*, but its abundance on *T. angustifolia* and the hybrid were not different. The moth *D. julianalis* was also found in highest

abundance on *T. latifolia*, but it is not found at all on *T. angustifolia*. Neither case is necessarily inconsistent with the *L. phragmitella* pattern, since the differences in abundance between the hybrid and each parental could be governed by different processes. For instance, the depressed abundance on the hybrid compared to *T. latifolia* could be due to seed availability, whereas the lower abundance on *T. angustifolia* could be due to female oviposition preference for *T. latifolia* and the presence of *T. latifolia* host recognition characters in the hybrid. The case of *D. julianalis* is complicated by the fact that it is not feeding exclusively on seeds, but is in fact an omnivore (see Chapter 2).

Depressed abundance of *L. phragmitella* on *T.* × *glauca* is an important ecological consequence of *Typha* hybridization, especially since *T.* × *glauca* prevalence is increasing and it is considered an invasive species (Galatowitsch 1999). *Limnaecia phragmitella* appears to influence the abundance patterns of many other arthropods associated with *Typha* spp., particularly predators and parasitoids that use *L. phragmitella* (see Chapter 1). Its abundance makes it an ecological dominant insect in this system, and it may function as an ecosystem engineer if its actions in the seed head really do result in the plant retaining the seed-head fluff through the winter as suggested by Claassen (1921). A diverse assemblage of arthropods can be found overwintering in the puffy seed-heads of senesced cattails, including many fungus feeders and detritivores that could be feeding on *L. phragmitella* frass (see Chapter 1). *Limnaecia phragmitella* may also serve directly as an important winter food source for birds. A significant portion of *L. phragmitella* larvae burrow into the cattail stems in fall or winter, and stems containing larvae are frequently torn into by birds (pers. obs). Black-capped chickadees have also been observed in winter pulling apart the fluff of cattail heads and feeding on *L. phragmitella* larvae (pers. obs.).

It is not known whether *L. phragmitella* populations could be self-sustaining (albeit at low abundance) on *T.* × *glauca*, or whether they require constant influx of moths reared on *T. latifolia* and *T. angustfiolia* plants. If the latter is true, then what threshold abundance of parental species is required to sustain *L. phragmitella* populations at different scales? Very little is known about *L. phragmitella* dispersal behavior, but it is possible that source-sink dynamics among cattail patches could occur, especially in regions where *T.* × *glauca* forms extensive monocultures. Where cattail species are intermingled, *T.* × *glauca* may function as an ecological trap for *L. phragmitella*. An ecological trap can result when habitat suitability and attractiveness become decoupled (Robertson and Hutto 2006), often as a result of habitat alteration on a short time scale. Though most often seen as the direct result of human activity, ecological traps could also be caused by the appearance of novel genotypes that result from interspecific hybridization. In this study, *L. phragmitella* females did not avoid ovipositing on *T.* × *glauca* despite our evidence that it does not provide an adequate supply of seeds to support many larvae. With respect to *T. latifolia*, the hybrid may represent an "equal-preference trap" (Robertson and Hutto 2006). The preference tests in this study showed some evidence (albeit not strong) that moths prefer *T.* × *glauca* to *T. angustifolia*; if they actually do prefer the less suitable host, then *T.* × *glauca* could represent a "severe trap" (*sensu* Robertson and Hutto 2006) with respect to *T. angustifolia*. However, the effect of *T.* × *glauca* presence on moth fitness could be mitigated because females lay eggs singly or in small groups and often utilize more than one host plant (pers. obs.).

In summary, we have shown that low abundance of *L. phragmitella* on hybrid cattails is probably a question of food limitation, since the hybrid produces very low amounts of seed compared to the parental, and the differences in seed abundance mirror the differences in *L.*

phragmitella biomass produced on parental vs. hybrid cattails. Although we cannot rule out a role for other mechanisms affecting *L. phragmitella* abundance, the seed supply problem appears sufficient to explain the overall pattern. As an example of hybrid resistance, this case appears to be an anomaly, since the insect response is probably not the result of plant resistance traits. However, the phenomenon might be expected given the feeding mode of the herbivore and the frequency of seed deficiencies in hybrid plants. This also appears to be a case of preference/performance mismatch; despite poor larval performance on *T.* × *glauca*, female *L. phragmitella* show no strong oviposition preferences for parental cattails, implying that increasing *T.* × *glauca* prevalence may result in a decrease in *L. phragmitella* abundance at a landscape scale.

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APPENDIX

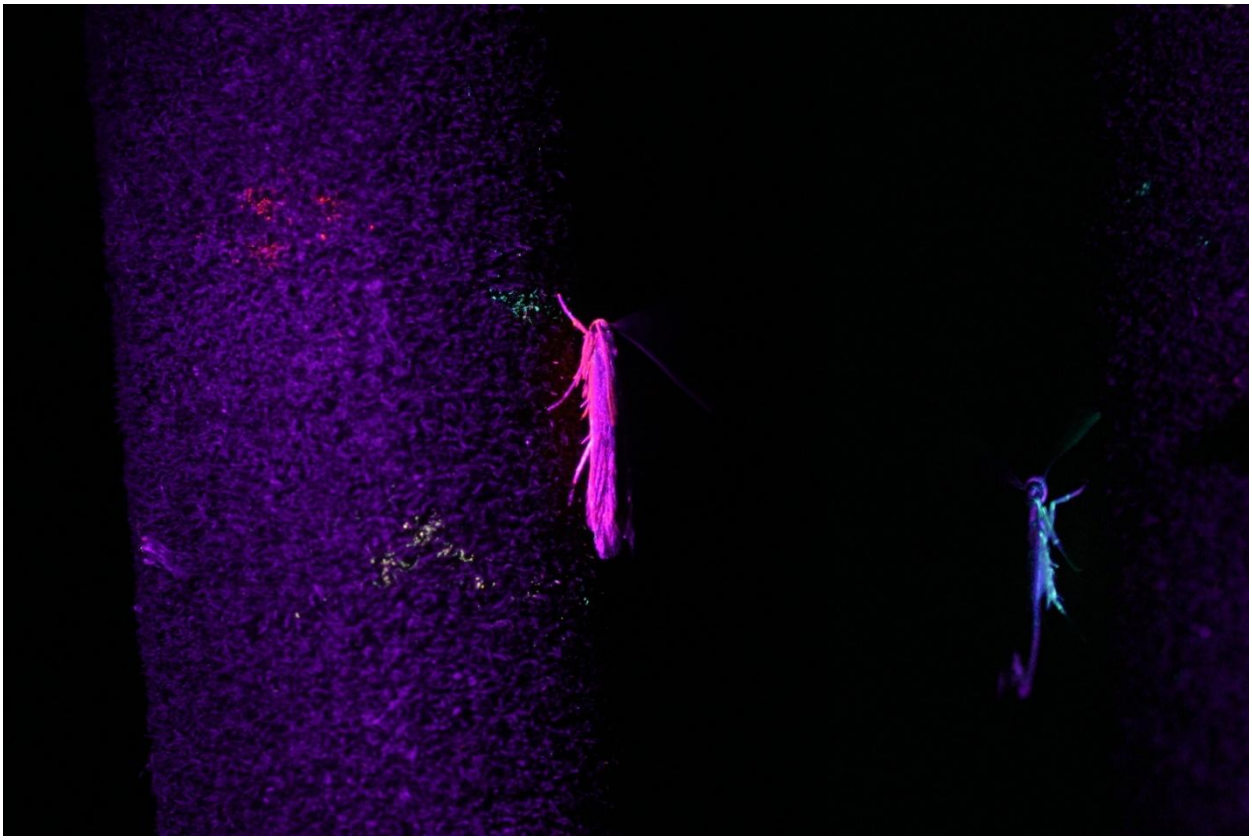


Figure 3.A1. Adult female *L. phragmitella* dyed with fluorescent powder and glowing under a blacklight (on cattail heads). On the left-hand cattail, smudges of fluorescent powder from other moths (yellow, green) are clearly visible.

Supplementary Genotyping Results

Out of 152 shoots genotyped, we found 65 genets (unique genotypes from a given site). We found 5 to 6 alleles at each locus (Table A1). Alleles were all species-specific, and allele sizes were largely consistent with those reported by other researchers (Tsyuko-Omeltchenko et al. 2003, Snow et al. 2010, Travis et al. 2010, Kirk et al. 2011). Some discrepancies in allele size/frequency are expected since the studies were conducted in different geographical regions.

Table 3.A1. Allele sizes and numbers of alleles found in *T. angustifolia* (26 genets), *T.* × *glauca* (14 genets), and *T. latifolia* (25 genets). All *T.* × *glauca* genets were F1s.

Locus 3			
Allele size (bp)	TA	TG	TL
174			15
176		14	24
178			6
180			5
209	48	14	
215	4		

Locus 5			
Allele size (bp)	TA	TG	TL
278			5
280		13	36
282		1	9
288	26	2	
290	5	4	
294	21	8	

Locus 7			
Allele size (bp)	TA	TG	TL
190		13	47
192		1	2
194			1
196	42	12	
209	10	2	

Locus 8			
Allele size (bp)	TA	TG	TL
270			10
272		14	40
276	17	4	
290	4	4	
292	31	6	

Locus 16			
Allele size (bp)	TA	TG	TL
178			2
180		11	32
182		3	13
184			3
194	47	14	
196	5		

Locus 20			
Allele size (bp)	TA	TG	TL
92		13	49
94		1	1
100	31	2	
102	8	6	
104	13	6	

CHAPTER 4

FIELD IDENTIFICATION OF *TYPHA LATIFOLIA, T. ANGUSTIFOLIA*, AND THEIR F1 HYBRID *T.* × *GLAUCA* BASED ON MORPHOLOGICAL ANALYSIS OF GENETICALLY-IDENTIFIED CATTAILS

ABSTRACT

Cattails (*Typha* spp.) are important wetland plants that are the subject of extensive research in many areas of biology and environmental science. Accurate identification of *Typha* species can be essential, but frequent interspecific hybridization makes identification complicated. In the United States, *T. latifolia* hybridizes with *T. angustifolia* to produce a distinct form called *T.* × *glauca*. DNA analysis has shown that most hybrid cattail plants in natural stands are F1 generation hybrids, but introgression can occur and the prevalence of advanced-generation hybrids (F2s, backcrosses, etc) varies considerably. Previous studies have emphasized the necessity of using DNA analysis to distinguish *T. latifolia* and *T. angustifolia* from their hybrids, but for many researchers and managers, these techniques are unavailable or impractical. In this study, we examined morphological characters of genetically-identified cattails from a variety of sites in central New York State. We identified shoots in the field as *T. latifolia*, *T. angustifolia*, or *T.* × *glauca* based on gross morphological characters, and then genotyped them using microsatellite DNA analysis. All the shoots designated *T.* × *glauca* in the field were shown to be F1 hybrids, and all the shoots designated as the parental species were correct except for one advanced hybrid genotype that was indistinguishable from *T. angustifolia*. We conclude that the F1 hybrids of *T. latifolia* and *T. angustifolia* are readily distinguishable from the parental species using field characters. Second generation or backcross individuals are probably indistinguishable

from the parental species, but these are not common in all locations, and for some research questions, distinguishing them may be relatively unimportant. We present a novel key to some useful qualitative characters that reliably separate the parental from the F1 forms, and we show how discriminant analysis can be used as a tool to separate the forms using quantitative characters.

INTRODUCTION

Cattails (*Typha* spp.) are widespread and important emergent macrophytes that can be ecologically dominant in wetlands worldwide. *Typha* is the subject of extensive research in many areas of biology and environmental science, including invasion biology, interspecific hybridization, wetland restoration, constructed wetlands and wastewater treatment, and nutrient cycling in wetlands. Accurate identification of *Typha* species can be essential for management as well as research, especially since some cattail species are considered invasive and misidentification could compromise restoration efforts or control programs. Cattail identification can be complicated by the presence of extensive phenotypic variation within taxa, and the possibility of interspecific hybridization. Molecular markers are available but not practical for many workers needing accurate identifications. In this report we evaluate morphological characters in genetically-identified cattail plants in order to help clarify questions of field identification and hybrid prevalence in northeastern North American cattails.

Two species of cattail exist in northern North America: the broad-leaved cattail *T. latifolia*, and the narrow-leaved cattail *T. angustifolia*. *Typha latifolia* is native, but the geographic origin of *T. angustifolia* is controversial. It was not mentioned in the earliest floras from North America, and when it was reported in 1820 it was apparently most common in

coastal areas (Stuckey and Salamon 1987). However, recent paleobotanical evidence from pollen deposition indicates that this species was present in North America long before European colonization, though not widespread (Shih and Finkelstein 2008). In either case, in the last 200 years *T. angustifolia* has undergone a westward range expansion into areas of North America where it was not historically present (Hotchkiss & Dozier 1949, Grace and Harrison 1986, Galatowitsch et al. 1999, Smith 2000, Shih and Finkelstein 2008). Throughout its range, it hybridizes with *T. latifolia* to produce a distinct form known as *T.* × *glauca*, which is considered invasive because of its tendency to displace other wetland vegetation and produce monocultures via rhizomatous growth (Smith 1987, Galatowitsch et al. 1999, Smith 2000, Zedler and Kercher 2004). All cattails have some tendency to create monospecific stands, but *T.* × *glauca* appears to pose more of a problem than *T. angustifolia*, and *T. latifolia* often coexists with other wetland species (Grace and Harrison 1986).

The genetic status of *T.* × *glauca* has long been the subject of debate. It was first described in France by Godron (1844) as the distinct species *T. glauca* (Hotchkiss and Dozier 1949; includes text and translation). Subsequently, several European authors suggested that *T. glauca* could be a *T. latifolia* × *T. angustifolia* hybrid, but most continued to regard it as a separate entity (reviewed by Hotchkiss and Dozier 1949 and Smith 1967). Dudley (1886) described a form in NY State he called *T. latifolia* var. *elongata*, and Wiegand (1924) renamed it *T. angustifolia* var. *elongata* based on perceived greater similarity to *T. angustifolia.* Hotchkiss and Dozier (1949) equated *T. angustifolia* var. *elongata* with *T. glauca* Godr. Smith (1967) performed experimental crosses and related the morphologies of the F1s to natural *T. glauca*, as well as the published descriptions of *T. glauca, T. angustifolia* var. *elongata*, etc., concluding

that the distinct form now known as *T.* × *glauca* is primarily an F1 (first generation) hybrid between *T. latifolia* and *T. angustifolia*.

As molecular markers have become more cost-effective and reliable, a number of studies have applied molecular techniques (primarily RAPD and microsatellite DNA) to the problem of identifying hybrid cattails and detecting introgression from one parental species into another (Kuehn et al. 1999, Tsyuko-Omeltchenko et al. 2003, Selbo and Snow 2004, Olson et al. 2009, Travis et al. 2010, Snow et al. 2010, Kirk et al. 2011). Kuehn et al. (1999) conducted a broad survey of cattails in northern North America, sampling in the Great Lakes Basin, Ontario, New York, Manitoba, Quebec, and Massachusetts. All hybrids found in this study were F1s, and the authors concluded that advanced-generation hybrids (backcrosses to parental species, F2s (F1 × F1), and more complex crosses) are rare in nature, though they can be produced experimentally and grown from field-collected *T.* × *glauca* seed. However, several more recent studies have reported advanced generation hybrids in natural populations. Snow et al. (2010) reported finding them in Michigan; Travis et al. (2010) found them in the Upper Midwest/Western Great Lakes region (Minnesota and Indiana); and Kirk et al. (2011) found them in Ontario, Quebec, Nova Scotia, and New Brunswick.

Nevertheless, it seems that advanced hybridization occurs in some areas more than others. Selbo and Snow (2004) found no hybrids at all in a wetland where *T. latifolia* and *T. angustifolia* were sympatric, despite overlap in flowering times and available substrate that appeared suitable for seedling germination. Snow et al. (2010) found advanced-generation and backcross hybrids in only 7 of 18 populations, even though they specifically targeted plants with phenotypes that were morphologically intermediate, with the goal of finding backcrossed and advanced-generation hybrids. Travis et al. (2010) found that F1 hybrids dominated all five of the

sites they sampled, with some advanced-generation and backcross genotypes present at all sites. The prevalence of these, however, varied considerably from one site to another. Kirk et al. (2011) also noted variation in the prevalence of advanced-generation and backcross hybridization among sites, with the Ontario locations having particularly high levels of introgression. No hybridization at all was found at the two sites they sampled in Maine (though only *T. latifolia* was present at those sites). Olson et al. (2009) sampled cattails from 40 sites along a transect of approximately 2000 km in southeastern Canada, and found only parental and F1 genotypes.

While molecular techniques have advanced our ability to study hybridization in cattails, they are expensive and time-consuming to use, and many researchers and managers working with cattails rely on morphological characters for identification. Do the recent reports of advanced hybridization in some cattail populations invalidate the use of morphology for identification of cattails? Many botanists have described the morphology of *T. latifolia* and *T. angustifolia* and discussed characters that are potentially useful in distinguishing these from the form called *T.* × *glauca* (Hotchkiss and Dozier 1949, Smith 1967, 1987, 2000; Tompkins and Taylor 1983, Grace and Harrison 1986, Gertz et al. 1994, Kuehn and White 1999, Snow et al. 2010). Two studies (Kuehn and White 1999, Snow et al. 2010) have analyzed morphological characters of genetically-identified cattails to determine whether field characters can be used successfully to identify hybrids. Kuehn and White concluded that discriminant analysis on a suite of quantitative traits was necessary to distinguish F1 hybrids from the parental species, because while hybrids tended towards intermediacy, there was significant overlap between hybrids and parentals for all traits they measured. The set of traits they recommended using in discriminant analysis included stigma width, which must be measured under the microscope. Snow et al.

(2010) performed a linear discriminant analysis that used a more practical set of traits, and employed allometric scaling of some traits to account for variation in plant size regardless of genotype. They concluded that "researchers familiar with morphological variation in *Typha" may* be able to separate F1 from parental cattails in the field, but that introgressed individuals would not be distinguishable. The variables used in the analysis were described, including the most predictive variable, which was an allometrically-scaled transformation of leaf width. However, actual trait values of genetically identified cattails were not included in the report, as the intention was to demonstrate the utility of microsatellite markers for identifying cattail hybrids in North America. At the present time there are very few resources available to help *Typha* workers make better field identifications or assess the validity of their field identifications in the face of hybridization.

We conducted a study of genetically-identified cattails from the area surrounding Ithaca, NY, USA, to determine whether gross morphological characters (including some not used by Kuehn and White (1999) or Snow et al. (2010) can be used successfully for field identification of hybrid cattails in this region. We go further than Snow et al. (2010) by concluding that F1 hybrids are readily distinguishable from parental species using field characters, as long as introgression is not common in the target populations, and we present practical methods of identification for scientists and managers working with cattail.

METHODS

Morphological measurements

Cattails were sampled from Ithaca, NY and nearby areas. Plants were collected in 2008 from 12 sites representing a variety of habitats, including marshes, wet meadows, lake edges,

small ponds, and roadsides. We defined a "morphotype" as a phenotypically-distinct form growing in a single location. Cattail morphotypes were identified as putative *T. latifolia* (TL), *T.* × *glauca* (TG), or *T. angustifolia* (TA). In this study, a morphotype by definition could occur only at one site, but some sites had several morphotypes of the same putative species. The total number of morphotypes from which samples were taken (across all sites) was 10 *T. latifolia*, 12 *T. angustifolia*, and 9 *T.* × *glauca*. Three flowering shoots were randomly chosen to represent each morphotype. Because *Typha* reproduces vegetatively via rhizomatous growth, we sampled shoots several meters apart to maximize the number of unique genotypes sampled from each morphotype. The morphological characters measured/observed for this analysis (Table 4.1, Figure 4.1, and Figure 4.2) included quantitative characters, many of which have been shown previously to vary across *Typha* taxa, and three qualitative characters which have not until now been defined clearly enough to be used for identification purposes.

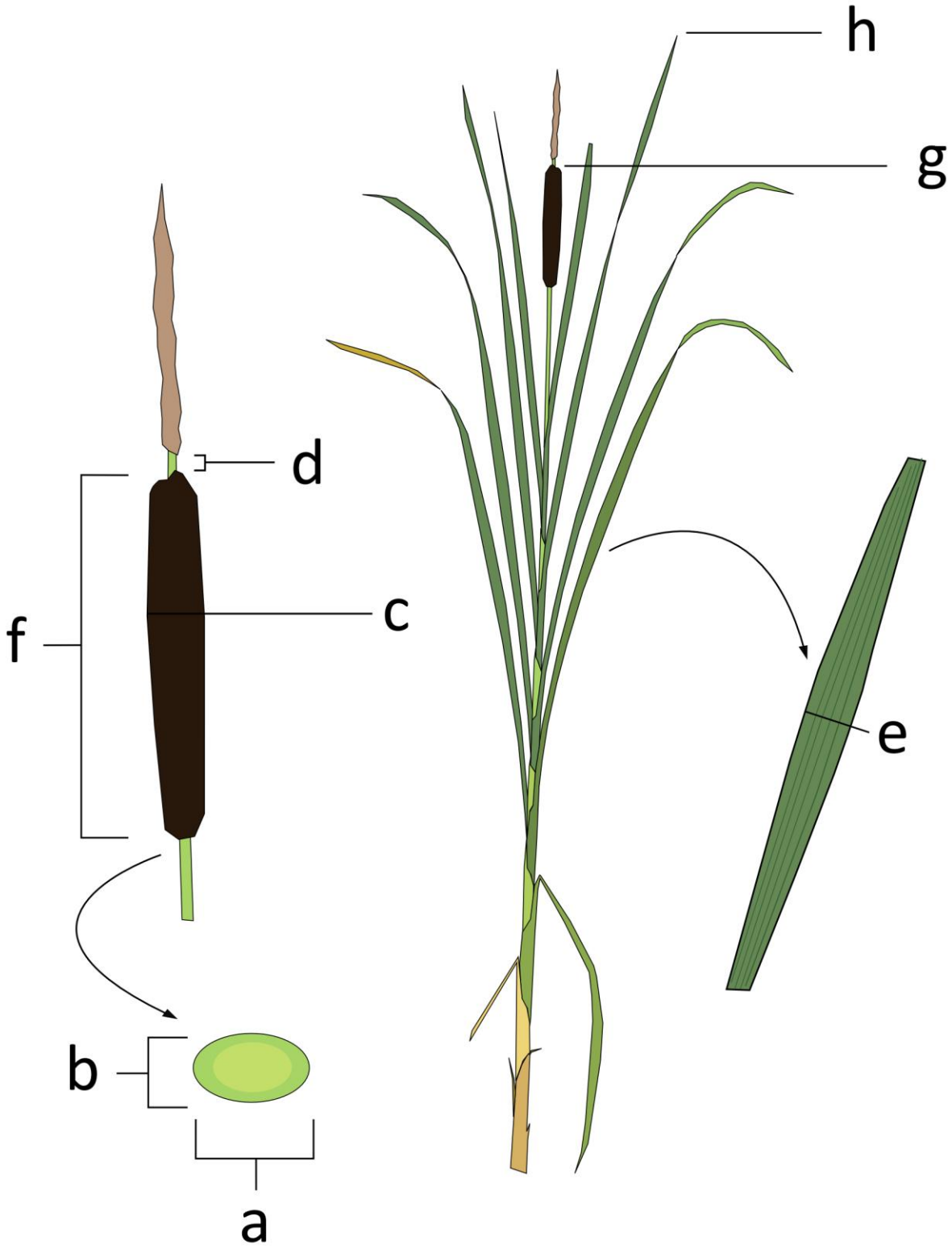
Table 4.1. Morphological characters that were measured/observed for each shoot in this study. The primary characters are illustrated in Figures 4.1 and 4.2. The last columns indicate (from Results section) whether the measurement needs to be taken in order to use our primary discriminant analysis (DA), discriminant analysis based on senesced shoots (S-DA), or key.

				Needed for:		
Field character	**Units**	**Tool**	**Figure**	**DA**	**S-DA**	**Key**
Maximum diameter of stem just below pistillate spike	mm	calipers	4.2a	✔	✔	
Minimum diameter of stem just below pistillate spike	mm	calipers	4.2b	✔	✔	
Maximum diameter of stem at the base (ground level)	mm	calipers				
Minimum diameter of stem at the base (ground level)	mm	calipers				
Maximum spike width	mm	calipers	4.2c	✔		
Minimum interval length, or distance between staminate and pistillate spikes	mm	calipers	4.2d	✔	✔	
Maximum leaf width	mm	ruler	4.2e	✔		
Maximum spike length	cm	ruler	4.2f		✔	
Shoot height from the ground to the top of the pistillate spike (= "head height")	cm	tape measure	4.2g	✔	✔	
Shoot height from the ground to the tip of the tallest leaf (= "leaf height")	cm	tape measure	4.2h	✔		
Amount of twist (helical rotation) in the tallest leaf (90° increments)	degrees	by eye				
Color of emerging pistillate spike (green or brown)		by eye	4.1 A, B, C			✔
Texture of midsummer pistillate spike (shaggy or not)		by eye	4.1 D, E, F			✔
Color of midsummer spike (having a blackened, toasted appearance or not)		by eye	4.1 G, H, I			✔

Figure 4.1. Photographs illustrating the qualitative color/texture characters used in this study. The first row shows the color of the pistillate spike at the onset of anthesis for *T. angustifolia* (A, brown), *T.* × *glauca* (B, green), and *T. latifolia* (C, green). The *T. angustifolia* photo shows a plant that is nearing the end of flowering but can still be scored for this trait. Note that on this shoot the stigmas at the top of the pistillate spike (1) are beginning to dry and turn brown themselves, but the stigmas at the bottom of the pistillate spike (2) are still whitish and fresh, and the brown color beneath them (a result of brown-tipped pistillate bracteoles) is the character in question. The second row shows the texture of the midsummer pistillate spike for *T. angustifolia* (D, shaggy), *T.* × *glauca* (E, not shaggy), and *T. latifolia* (F, not shaggy). The third row shows the color of the midsummer pistillate spike for *T. angustifolia* (G, cinnamon), *T.* × *glauca* (H, cinnamon), and *T. latifolia* (I, blackened). The colors are also visible in the close-up photos in the second row. Photos by S. Reilly.


A
1
2
B
C
D
E
F
G
H
I

Figure 4.2. Diagram of a cattail plant indicating the morphological traits measured for this analysis. (a, b) represent maximum and minimum diameters of the stem just below the pistillate spike (a cross section of stem is shown). (c) is the maximum width of the pistillate spike. (d) is the minimum distance between pistillate and staminate spikes; this portion of bare stem is often asymmetrical in length. (e) is the maximum width of the widest leaf. (f) is the maximum length of the pistillate spike. (g) is the height from the ground (even if under water) to the top of the pistillate spike (= "head height"). (h) is the height from the ground to the tip of the tallest leaf (= "leaf height"). Hold the leaf straight up while measuring. The units of measurement and the recommended tools are presented in Table 4.1.


h
g
d
c
f
e
b
a

Initial morphological observations were made in early summer when the plants were just beginning to flower, so that the initial color of the pistillate spike could be observed. Shoots were tagged with flagging, and a leaf sample was collected and frozen at -20°C for genotyping. Plants were revisited later in the summer when pistillate spikes were full-sized, and the remaining morphological characters were observed at that time. In a few cases, the tagged shoot was missing or damaged, so a replacement shoot was chosen as near as possible to the original shoot, and a new leaf sample taken for genetic analysis.

Discriminant analysis

The quantitative field characters (Table 4.1) were used to create a set of trait variables for discriminant analysis (Table 4.2). Several characters (stem diameter measures, spike width, spike length, leaf width, leaf overshoot) varied with plant size independent of plant species, which can interfere with their ability to discriminate taxa especially since all quantitative traits used here show some overlap among taxa (e.g. a small *T. latifolia* shoot may have leaves that are similar in width to those of a large *T.* × *glauca*). Therefore, these traits were allometrically scaled by a variable representing overall plant size. In the case of leaf width, for example, scaling produces a new variable representing how wide the leaves are for the size of the plant. We scaled by dividing trait values by the height of the shoot to the top of the pistillate spike ("head height"). Scaling by the height of the shoot to the tip of the tallest leaf was another option, but the head height is a more reliable field character for general use because leaves can be broken off or damaged by herbivores. Within a plant species, these two height measures are highly correlated in our dataset.

Table 4.2. Trait variables created for discriminant analysis (before model selection). Stem roundness was calculated as the difference between the maximum and minimum stem diameters for each shoot, divided by the average diameter for that shoot, i.e. roundness = 1– (*max stem diameter – min stem diameter*)/(*average stem diameter*).

Variable	Description	Scaled
maxstem_H	maximum stem diameter just below spike	✔
maxstem_B	maximum stem diameter at base (ground level)	✔
stemdiameter_H	average of max and min diameters of stem just below spike	✔
stemdiameter_B	average of max and min diameters of stem at base (ground level)	✔
roundness_H	index of stem roundness immediately below the pistillate spike	
roundness_B	index of stem roundness at base (ground level)	
overshoot	difference between head height and leaf height	✔
leafwidth	maximum leaf width	✔
interval	distance between staminate and pistillate spikes	
headwidth	width of the full-sized pistillate spike	✔
headheight	distance to the top of the pistillate spike	
headlength	maximum spike length	✔
leafheight	distance to the tip of the tallest leaf	
rotation	amount of twist (helical rotation) in the tallest leaf (90 ^{o} increments)	

These variables (Table 4.2) were used to perform a linear discriminant analysis in R (R Development Core Team 2012) using the lda() function in the MASS package (Venables and Ripley 2002). We used forward stepwise model selection based on the Wilks' lambda criterion, with a threshold p-value for variable inclusion of 0.05. In addition to the primary discriminant analysis which included all quantitative variables, a separate discriminate analysis was performed on the subset of variables that would be measurable on senesced plants, to determine whether field identification of senesced plants using these traits is possible.

Genotyping

All shoots from which morphological measurements were taken were genotyped using microsatellite markers to verify parental vs. hybrid status. Plant tissue was collected in early summer and stored at –20°C. Tissue was lyophilized and then ground using a GenoGrinder. Ground tissue was returned to –20°C until extraction. DNA was extracted with plant DNeasy kits

(Qiagen, Inc. City State), using the basic miniprep protocol. We used 6 primers developed for one of the parental cattail species, *T. angustifolia* (TA3, TA5, TA7, TA8, TA16, TA20) (Tsyuko-Omeltchenko et al. 2003) and tested in North American cattails (Snow et al. 2010). These primers amplify in *T. latifolia* as well, and appeared likely to amplify distinct alleles in the two parental species in our study populations. F1 individuals would be characterized by having one allele from each parental species at each locus. Advanced hybrids and backcrosses would display a combination of species-specific loci and mixed-species loci. The probabilities of misidentifying F2 or first-generation backcrosses using 6 loci, assuming Mendelian inheritance, are given in Table 4.3. Based on these probabilities, we determined that using 6 alleles was sufficient.

Table 4.3. Probabilities associated with misidentification of backcross or F2 individuals based on 6 microsatellite loci.

Actual Genotype	Apparent Genotype	Probability
Backcross	Pure parental	0.016
Backcross	F1	0.016
F2	Pure parental	0.0005
F2	F1	0.016

The 5' end of each primer was fluorescently labeled with NED (TA3, TA20), VIC (TA5, TA16), 6FAM (TA7) or PET (TA8). PCR was performed using Multiplex PCR kits (Qiagen, Inc. City State), using the standard protocol with Q solution, except that the ratios of the primers in the primer mix were optimized (volume per reaction was increased from 0.2 to 0.3 µl for TA5, and decreased to 0.15 for TA16 and TA20). PCR was performed using the following cycling parameters: 95° for 15 min; 7 x (94° C for 30s, 57° C for 1 min 30s (-1° C per cycle)); 72° C for 1 min; 25 x (94° C for 30s, 50° C for 1 min 30s, 72° C for 1 min; 72° C for 10 min. Genotyping was performed using an ABI capillary sequencer in the Evolutionary Genetics Core Facility at

Cornell University. Data were collected and scored manually using Genemapper v. 3.0 (Applied Biosystems).

RESULTS

Genotyping revealed no evidence of introgression among plants identified in the field as *T. latifolia*. All putative *T. latifolia* plants contained TL-specific alleles at all 6 loci. All plants identified in the field as *T.* × *glauca* showed a classic F1 genotype pattern, having one TL allele and one TA allele at each locus. One morphotype that was identified as *T. angustifolia* in the field was actually an advanced hybrid, probably a backcross to *T. angustifolia*. This morphotype was homozygous for TA alleles at locus 5 and locus 20, but contained both TL and TA alleles at the remaining loci. There was no evidence of introgression in any other putative *T. angustifolia* morphotypes.

A total of 31 morphotypes were sampled (see Methods). In some cases, shoots sampled from the same morphotype displayed the same alleles at all 6 loci, and were probably clones. In many instances, however, at least one of the shoots sampled from a single morphotype represented a different genet. Overall, about half of the morphotypes (17/31) represented more than one genet. Of 101 total shoots genotyped, we found 55 genets. We found 5 to 6 alleles at each locus (Table 4.4). Alleles were all species-specific, and allele sizes were largely consistent with those reported by other researchers (Tsyuko-Omeltchenko et al. 2003, Snow et al. 2010, Travis et al. 2010, Kirk et al. 2011). Some discrepancies in allele size/frequency are expected since the studies were conducted in different geographical regions.

Table 4.4. Allele sizes and numbers of alleles found in *T. angustifolia* (24 genets), *T.* × *glauca* (14 genets), and *T. latifolia* (16 genets). All *T.* × *glauca* genets were F1s. The backcross to *T. angustifolia* (BA) genet was included separately.

Locus 3				
Allele size (bp)	TA	BA	TG	TL
174				12
176		1	13	15
178			1	3
180				2
209	47	1	14	
215	1			

Locus 5				
Allele size (bp)	TA	BA	TG	TL
277				2
279			13	26
281			1	4
288	24		7	
290	10		1	
294	14	2	6	

Locus 7				
Allele size (bp)	TA	BA	TG	TL
190		1	14	25
192				4
194				3
196	33	1	10	
209	15		4	

Locus 8				
Allele size (bp)	TA	BA	TG	TL
270			2	4
272		1	12	28
276	23		6	
290			3	
292	25	1	5	

Locus 16				
Allele size (bp)	TA	BA	TG	TL
180		1	10	21
182			4	8
184				1
186				2
194	44	1	11	
196	4		3	

Locus 20				
Allele size (bp)	TA	BA	TG	TL
92			12	32
94			2	
100	22		4	
102	9	2	5	
104	17		5	

Typha x glauca (F1) plants were intermediate for some morphological traits, but resembled one or the other parental species for others (see Figure 4.3).

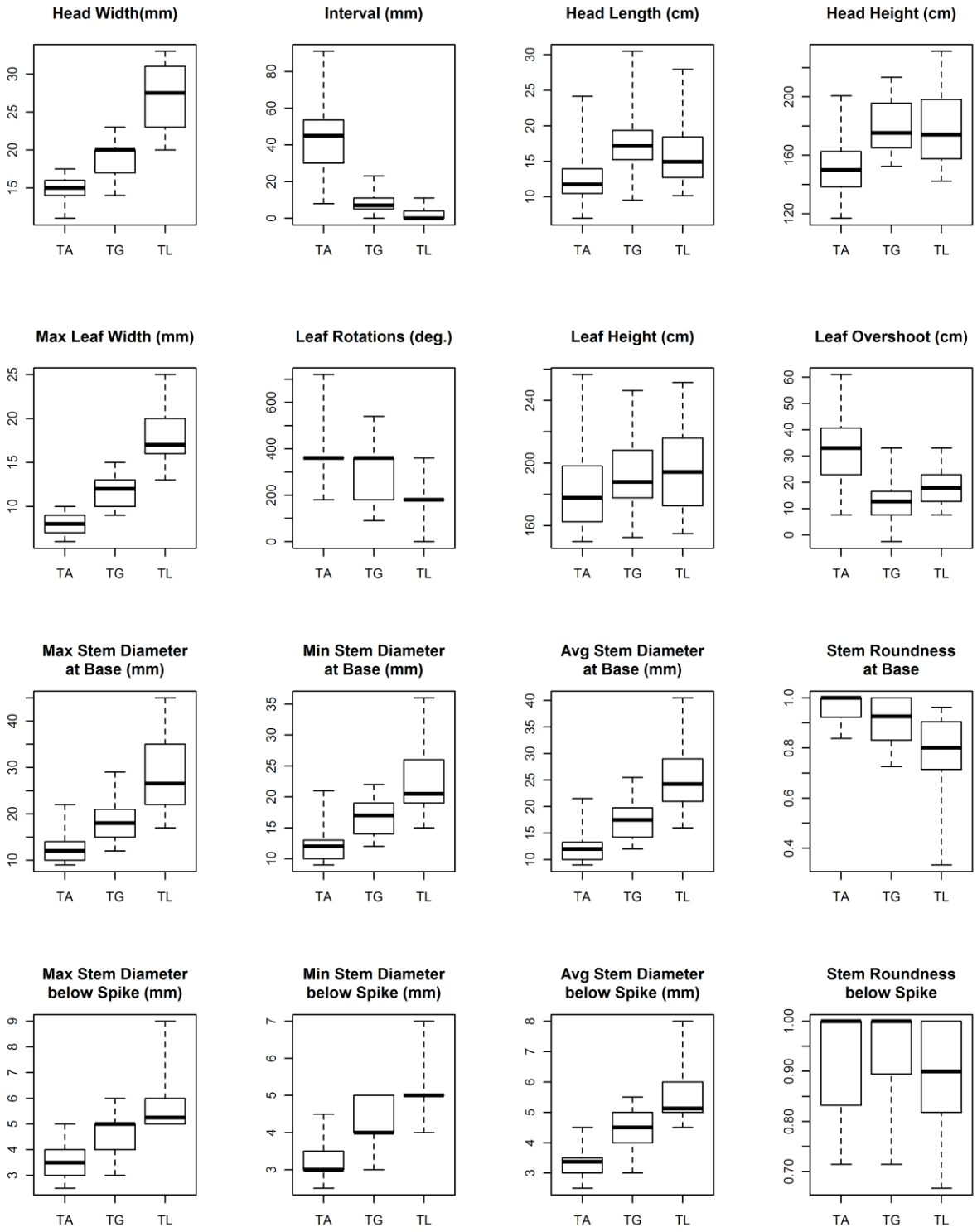


Figure 4.3. Boxplots showing trait values for genetically-identified *T. angustifolia* (TA), F1 *T.* × *glauca* (TG), and *T. latifolia* (TL). Traits are given as field values and are not allometrically scaled. Whiskers indicate maximum and minimum values.

Discriminant analysis was performed using data from genetically-identified *T. latifolia*, *T. angustifolia*, and F1 *T.* × *glauca* shoots. All shoots sampled were included in the analysis even if genotyping revealed them to be likely clones, since morphology differed considerably even within a clone. We did not include data from the single backcross genotype that we found (3 shoots with same genotype). Model selection resulted in a model that included the following six variables: *leafwidth*, *overshoot*, *interval*, *headwidth*, *headheight*, *roundness_H.* These variables are listed in the order of inclusion, with *leafwidth* being the most significant variable (see Table 4.5). Forward stepwise model selection acts by adding variables to the model in order of significance, such that at each step the variable that adds the most discriminatory power (given the other variables already in the model) is included. Variables need not discriminate all three species very well to be included in the model, since variables are chosen at each step which provide the most non-redundant information. For example, the variable *roundness_H* does not distinguish *T. angustifolia* and *T.* × *glauca* very well, but was chosen at the last step of model selection because it added to the model's ability to discriminate *T. latifolia* from the other two taxa. The variables included in the model work together as a set, and are not necessarily useful independently.

Table 4.5. Results of stepwise model selection

Variable	Wilks' lambda	F value for difference	p value for difference
Leafwidth	0.1912	181.8996	<0.001
Overshoot	0.0948	43.1965	<0.001
Interval	0.0668	17.6011	<0.001
Headwidth	0.0558	8.2254	<0.001
Headheight	0.0401	16.0169	<0.001
Roundness_H	0.0370	3.4288	0.037

This discriminant analysis correctly identified shoots in 98.9% of cases (one *T. angustifolia* plant was classified as a *T.* × *glauca*). The three backcross shoots were added to the discriminant function plot (Figure 4.4) to show that they would be grouped with *T. angustifolia*. This is consistent with our field identifications, as the shoots were originally classified as *T. angustifolia* based on field traits (quantitative and qualitative).

Other *Typha* shoots can be classified by using the classification functions generated by this analysis, as long as the same traits are measured using the same units. Classification functions are equations that use the trait values of an unidentified plant to generate a score (S) for each of the possible classifications (*T. latifolia, T. angustifolia*, or *T.* × *glauca*), the highest of which indicates the most likely classification. Classification functions for this discriminant analysis are:

$$S_{TA} = -176.938 + 956.068\,(leafwidth) - 17.274\,(overshoot) + 0.297\,(interval) + 639.218\,(headwidth) + 0.831\,(headheight) + 114.990\,(roundness)$$

$$S_{TG} = -225.591 + 1215.516\,(leafwidth) - 72.673\,(overshoot) + 0.090\,(interval) + 717.209\,(headwidth) + 0.977\,(headheight) + 130.191\,(roundness)$$

$$S_{TL} = -303.007 + 1560.444\,(leafwidth) - 81.243\,(overshoot) + 0.081\,(interval) + 876.144\,(headwidth) + 1.101\,(headheight) + 136.859\,(roundness)$$

To classify a shoot, calculate the score (S) for each species and assign the shoot to the species with the highest score.

Discriminant analysis using the subset of traits that would be measurable on senesced shoots was less accurate, but still potentially useful as it correctly identified shoots in 93.3% of cases. Including traits that may or may not be measurable on senesced shoots (such as leaf width, since leaves are often missing or damaged on senesced plants) increased the correctness rate to 97.8%. See Appendix for further details on these discriminant analyses.

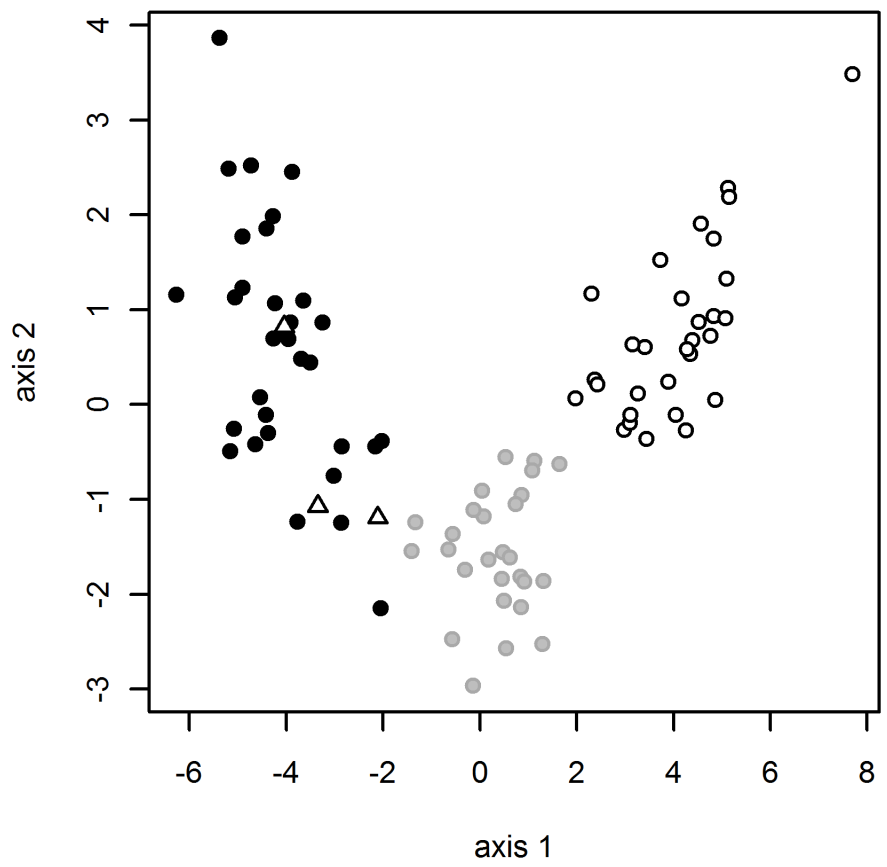


Figure 4.4. Discriminant plot with shoots labeled according to genotyping results. Open circles are *T. latifolia*, gray circles are *T.* × *glauca* (F1 hybrids), and black circles are *T. angustifolia*. Three shoots with an advanced hybrid genotype (backcross to *T. angustifolia*), are represented by open triangles. These shoots were not included in the discriminant analysis but would be classified as *T. angustifolia* based on the analysis.

Qualitative traits could not be included in the discriminant analysis, but are very important in making accurate and efficient field identifications. In this study, the three qualitative traits we employed (color of pistillate spike at onset of anthesis, color and texture of pistillate spike in midsummer) correctly identified 100% of parental and F1 cattails sampled for this study (advanced hybrids cannot be classified reliably using morphological characters). See Box 1.

Box 1. Simple key to *T. latifolia, T. angustifolia,* and *T.* × *glauca* (F1 only) based on qualitative traits.

1. Overall color of pistillate spike as it is emerging from the sheath (before or near onset of anthesis) (See Figure 4.1 A, B, and C).
    Brown[1]…….*T. angustifolia*
    Green……2

OR

Texture of midsummer (post-anthesis) pistillate spike (Figure 4.1 D, E, and F).
    Shaggy[2]…… *T. angustifolia*
    Not shaggy…….2

2. Overall color of midsummer (post-anthesis) pistillate spike (Figure 4.1 G, H, and I).
    Cinnamon with no black…….*T.* × *glauca*
    Having blackened or toasted appearance[3]……. *T. latifolia*

[1]The brown color of the emerging pistillate spike of *T. angustifolia* is due to the presence of dark brown pistillate bracteole tips that form the surface of the spike at this stage of development. At the onset of anthesis, the whitish-greenish stigmas grow past the bracts but the brown color can still be detected beneath them. Do not evaluate this character after the stigmas have begun to dry and turn brown themselves. At that point, the pistillate bracteoles can be seen in a cross-section of the spike as small black dots amongst the white fibers, at the surface of the spike but beneath the stigmas (Figure 4.5). *T. latifolia* lacks pistillate bracteoles and those of *T.* × *glauca* are inconspicuous and do not affect the color of the emerging spike.

[2] The "shaggy" appearance is due to the long, narrow stigmas of *T. angustifolia*, which hang down and often clump together on the surface of the spike when dry. This trait is somewhat time sensitive, as the stigmas can wear off by late summer.

[3]This trait is best observed in early or mid-summer, not late summer. The black color of *T. latifolia* is due to the dry stigmas, which wear off with time, leaving a more cinnamon-brown color that can be confused with *T.* × *glauca*. The black color appears immediately after anthesis, often resulting in a green spike with black highlights, later becoming brown with black highlights.

Figure 4.5. A broken pistillate spike from *T. angustifolia* in midsummer. The dark tips of the pistillate bracteoles can be seen as dark brown dots amongst the white fibers. Dried stigmas, in contrast, are elongated and cinnamon-colored. The left side of the photo shows the pistillate bracteoles in their natural positions just beneath the stigmas, which extend past the bracteoles. In the rest of the photo, the fluff has been dislodged to make the dark dots easier to see. *Typha latifolia* and *T.* × *glauca* (F1) would not show these dots.

DISCUSSION

The ecological and economic importance of *Typha* makes it a focal species in many research and management projects where accurate identification of taxa is necessary. Although interspecific hybridization can complicate field identification, our study shows that distinguishing *T. latifolia* and *T. angustifolia* from their F1 hybrids in the field is straightforward based on gross morphology of flowering shoots. It is likely that introgressed genotypes are indistinguishable from parentals and/or F1s without genetic analysis, but our study supports the idea that advanced hybridization is relatively rare at many sites. Therefore, the morphological traits we present in this paper can be used effectively in many circumstances to distinguish parental from F1 cattails, when DNA techniques are unavailable, or in combination with selective DNA analysis.

Previous studies have emphasized the difficulty of distinguishing hybrid cattails in the field, and encouraged researchers to rely on molecular techniques whenever possible. Those

without the resources or time available for DNA analysis are advised to use microscopic floral characters (Smith 2000) and/or discriminant analysis to distinguish cattail species (Kuehn and White 1999, Snow et al. 2010). Snow et al. 2010 implied that field identification of parental and F1 cattails may be possible by experienced researchers, but their report did not include data about the distributions of trait values from genetically-identified cattails that would help researchers gain confidence in their field identifications. Kuehn and White (1999) did provide data on trait values (range and mean), and also calculated the classification functions from their discriminant analysis, which theoretically makes it possible for other researchers to use their discriminant analysis as an identification tool. However, their analysis relies on a microscopic floral character, stigma width, which is not a practical trait for field identification, and is time-consuming to obtain in the laboratory. Even with stigma width included, their analysis does not differentiate parental and hybrid genotypes as well as that of Snow et al. (2010) or the analysis we report here. The better discrimination ability may be due in part to allometric scaling of some traits, which Kuehn and White (1999) did not do.

In areas where advanced hybridization is rare, the discriminant analysis presented in this paper can separate F1 individuals from the parental species with very good accuracy (98.9%), and the traits used are all easily obtainable in the field. This set was identified through model selection as the best combination of the variables we obtained, though many other combinations of traits can create discriminant analyses with similar classification ability. Researchers can use our discriminant analysis directly as an identification tool, by calculating classification scores for their own plants using the classification functions presented in this paper. While potentially useful in some scenarios, this tool must be used with caution. Our analysis may be less reliable for cattails in other regions, which could have trait values/combinations of traits that are outside

the ranges in our sample. Furthermore, our analysis is specific to northern North America where *T. latifolia* and *T. angustifolia* are the only cattail species. The traits presented here are not necessarily diagnostic in regions where *T. domingensis* and its hybrids occur. A discriminant analysis based on a larger sample of genetically-identified cattails from a wider geographic area would be useful, and this could be obtained collaboratively if researchers measured the same traits.

Qualitative traits are sometimes considered inferior to quantitative traits as identification characters (e.g. Kuehn and White 1999). In *Typha,* quantitative traits suffer from overlapping values across species, whereas some qualitative traits appear to be reliably diagnostic. The qualitative traits we present here (color of pistillate spike at the onset of anthesis, and color and texture of the midsummer pistillate spike) are holistic characters that reflect variation in floral characters. The brown color of the *T. angustifolia* spike at the onset of anthesis is due to the presence of dark-tipped pistillate bracteoles that form the surface of the spike before the stigmas elongate. *T. latifolia* lacks pistillate bracteoles, and those of *T.* × *glauca* are inconspicuous, so the color of their spikes is different at this stage. The color of the spike in midsummer after the stigmas have dried is a reflection of variation in stigma color, and the texture of the pistillate spike in midsummer is largely a function of stigma shape, which differs among *T. angustifolia*, *T. latifolia*, and *T.* × *glauca*. Kuehn and White (1999) quantified stigma width under the microscope, and it was the most significant predictive variable in their discriminant analysis. However, width alone does not capture the overall form of the stigma, and width values among taxa overlap. Using the qualitative color and texture characters facilitates better discrimination, and makes it possible to identify shoots at a glance in the field.

The validity of using these morphological traits to identify *Typha* depends on the frequency of advanced hybridization at the sites in question. The prevalence of introgression at different sites or in different regions appears to vary, with some studies reporting extensive introgression and others little to none (Kuehn et al. 1999, Olson et al. 2009, Snow et al. 2010, Travis et al. 2010, Kirk et al. 2011). In our study, all plants identified in the field as *T.* × *glauca* were shown to be F1 hybrids according to microsatellite DNA analysis. No plants identified as *T. latifolia* showed evidence of introgression, and only one morphotype identified as *T. angustifolia* in the field possessed alleles from both species and was probably a first generation backcross to *T. angustifolia*. In other studies (see Chapters 1 and 3), we genotyped an additional 370 shoots, some from the same sites included here, and some from 9 additional sites (all in the Ithaca, NY area), using the same genotyping protocols. We found no advanced hybrids or backcrosses. All field identifications of *T. latifolia*, and *T. angustifolia* were correct, and all shoots identified as *T.* × *glauca* were F1 hybrids.

In conclusion, the question of cattail identification boils down to distinguishing *T. latifolia* and *T. angustifolia* from their hybrids, since *T. latifolia* and *T. angustifolia* themselves are readily distinguished. The morphological form known as *T.* × *glauca* is probably characteristic of the F1 hybrid, and it is readily distinguished from the parentals using field traits as presented here. Unfortunately, the presence of introgression and advanced hybridization complicates identification, since these genotypes are probably not identifiable using morphological traits. However, the prevalence of introgression varies considerably, and may be unimportant in many cattail populations. Significant work has been done in recent years to establish good DNA markers for identifying cattail hybrids, and microsatellites now offer a relatively straightforward way to obtain accurate genotypes. *Typha* researchers whose

experiments involve hundreds or thousands of shoots cannot possibly genotype each one, especially if shoots are collected in a senesced state as would be the case for litter decomposition experiments. In that case, genotyping a subset may be useful for establishing the proportion of shoots that represent introgressed genotypes. Given the clonal nature of the plant, researchers in many cases could genotype a subset and then rely largely on morphotype, if the prevalence of introgression is low and/or the research question not very sensitive to the inclusion of a few introgressed individuals. For some studies, moving towards a trait-based approach (e.g. McGill et al. 2006) may be more appropriate than trying to classify each shoot by genotype, especially if introgression is rampant.

It is important to bear in mind that for some questions, classifying cattails into just three categories—*T. angustifolia*, *T. latifolia*, and *T.* × *glauca*—may be sufficient because it still provides useful information. For example, when documenting spread of the invasive *T.* × *glauca*, the most immediate question may be whether *T.* × *glauca* is actually displacing *T. latifolia* and/or *T. angustifolia* forms. Using field characters, researchers or managers can quickly and cheaply identify all the cattails at a given site and monitor changes in their distributions. Certainly this approach would be better than not making any such observations when funding for molecular techniques is unavailable. Field classification can be important even in studies that also utilize molecular markers, because making *a priori* species identifications often improves sampling efficiency and site selection. As molecular techniques for *Typha* identification have become more widely available, there is a tendency in the literature to overestimate the difficulty of making field identifications. In this paper, we show that distinguishing parental taxa and their F1 hybrids is not difficult, being complicated only by the presence of introgressed genotypes, which appears to be a problem at some sites and not at others.

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APPENDIX

A linear discriminant analysis was performed using only the subset of traits that would be available on senesced (but still standing) shoots. The variables included were:

headheight (distance to the top of the pistillate spike, not scaled)

headlength (length of the pistillate spike, scaled)

stemdiameter (average diameter of stem immediately below pistillate spike, scaled)

stemmax (max diameter of stem just beneath pistillate spike, scaled)

interval (length of gap between pistillate and staminate spikes, not scaled)

roundness (index of stem roundness immediately below pistillate spike, not scaled)

These variables are all measurable on senesced cattails, since the extent of the pistillate spike is visible on the axis even after the seeds and fluff have detached. Staminate spikes almost always break off after flowering, but the interval is still measurable because breakage usually occurs above the base of the staminate spike, the location of which is indicated by a scar on the axis.

Model selection resulted in a model including *interval*, *stemdiameter*, *headlength*, and *headheight* (Table 4.A1). Classification functions are as follows:

$$S_{TA} = -95.82 + 0.333\ (interval) + 4188.481\ (stemdiameter) - 383.197\ (headlength) + 0.763\ (headheight)$$

$$S_{TG} = -114.989 + 0.105\ (interval) + 4718.596\ (stemdiameter) - 451.302\ (headlength) + 0.876\ (headheight)$$

$$S_{TL} = -152.126 + 0.054\ (interval) + 5861.477\ (stemdiameter) - 632.752\ (headlength) + 1.003\ (headheight)$$

This model correctly identified shoots in 93.3% of cases, but there is significant overlap between F1 and parental groups (Figure 4.A1A).

Table 4.A1. Results of model selection

Variable	Wilks' lambda	F value for difference	p value for difference
Interval	0.3056	97.71	<0.001
Stemdiameter	0.2012	22.03	<0.001
Headlength	0.1607	10.60	<0.001
Headheight	0.1006	25.10	<0.001

A second analysis was performed using a set of variables containing the six above plus some that may or may not be available or reliable on senesced shoots:

leafwidth (max leaf width, scaled)

stembasediameter (average diameter at the base of the shoot, scaled)

stembasemax (max diameter at the base of the shoot, scaled)

stembaseroundness (index of stem roundness at the base of the shoot)

Model selection resulted in a model including *leafwidth*, *interval*, *headheight*, *headlength*, *stemdiameter*, *and roundness* (Table 4.A2). None of the characters relating to the base of the stem were significant, which is convenient because these are difficult or impossible to measure on shoots that are in deep water. This model correctly identified shoots in 97.8%.of cases (Figure 4.A1B).

Table 4.A2. Results of model selection

Variable	Wilks' lambda	F value for difference	p value for difference
Leafwidth	0.1911	181.90	<0.001
Interval	0.0983	40.09	<0.001
Headheight	0.0845	6.89	0.0017
Headlength	0.0748	5.41	0.0062
Stemdiameter	0.0620	8.42	0.0047
Roundness	0.0565	3.94	0.0233

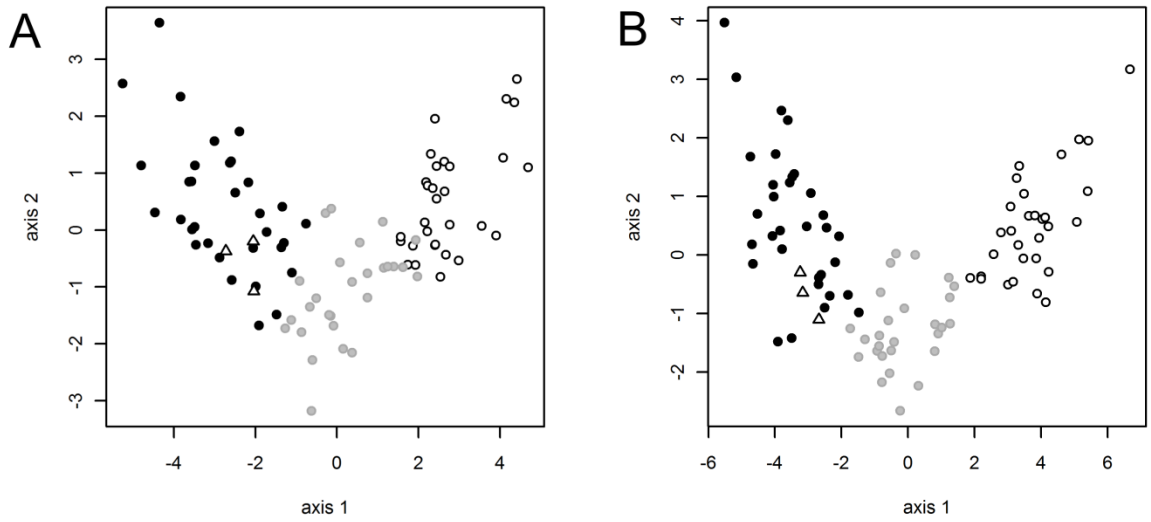

Figure 4.A1. Discriminant plots with shoots labeled according to genotyping results, for the first senesced shoot analysis (A) and the less-conservative option (B). Open circles are *T. latifolia*, gray circles are *T.* × *glauca* (F1 hybrids), and black circles are *T. angustifolia*. Three shoots with an advanced hybrid genotype (backcross to *T. angustifolia*), are represented by open triangles. These shoots were not included in the discriminant analyses but would be classified as *T. angustifolia* based on the analyses.

These analyses were performed to show that senesced shoots are often still identifiable, and discriminant analysis may be useful even though some of the better morphological characters are not available on senesced plants. The first analysis can be used as an identification tool based on the classification functions we provide, but bear in mind that it was performed using traits measured on flowering shoots, not senescent ones, and some characters shrink during senescence. The four traits used in the first analysis are probably not very subject to shrinkage, with the possible exception of stem diameter. The second analysis is more problematic because of the inclusion of leaf width, which is known to shrink. Thus, we did not include the classification functions for this analysis. A discriminant analysis using these traits appears potentially useful, but it would have to be created using traits actually measured on senesced plants.